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Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

OFFICIAL JOURNAL OF AMERICAN THORACIC SOCIETY
MEDICAL SECTION OF THE NATIONAL TUBERCULOSIS ASSOCIATION

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THE AMERICAN REVIEW OF RESPIRATORY DISEASES

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Medical Section of the National Tuberculosis Association

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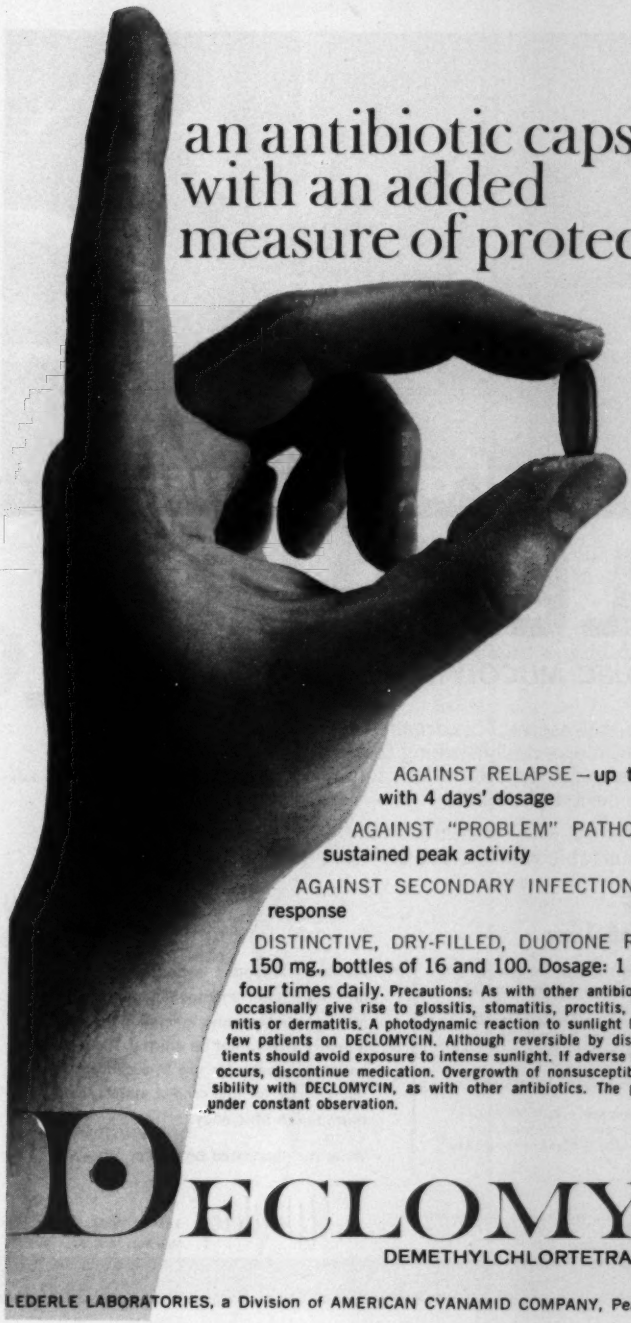

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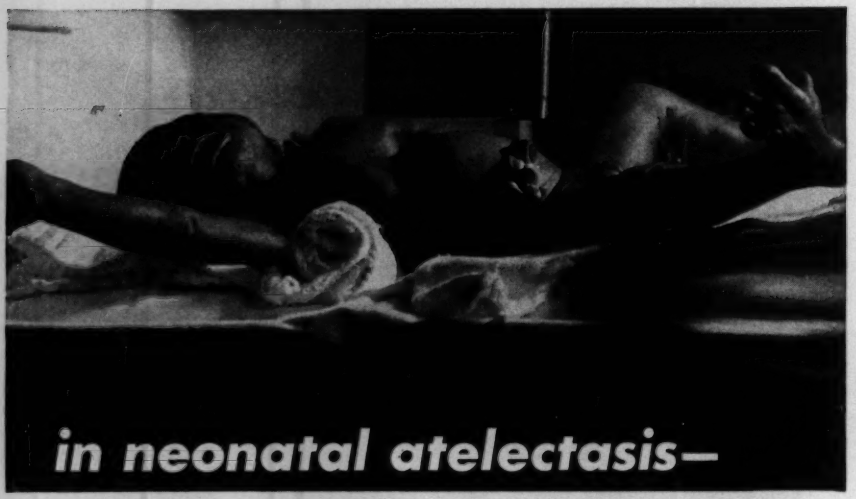

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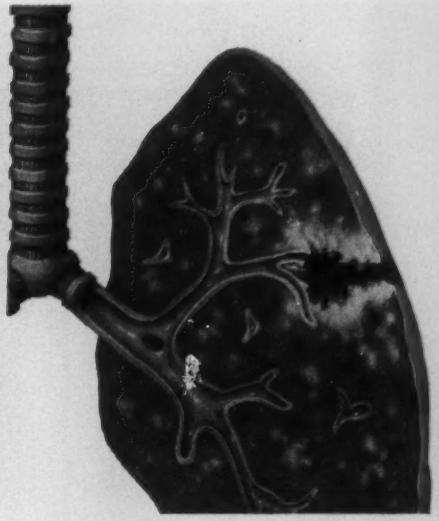

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1. Davey, W. N.: GP 19:107, No. 1, 1959.

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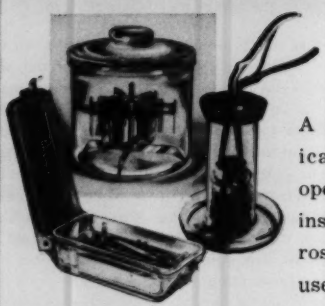

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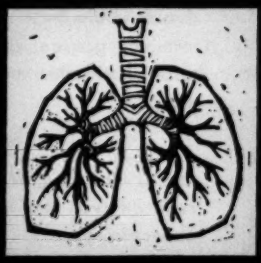

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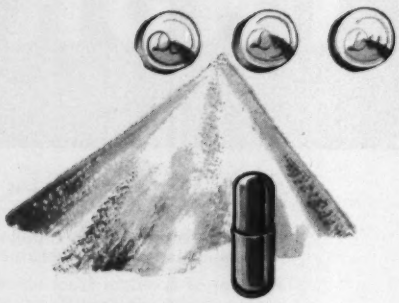

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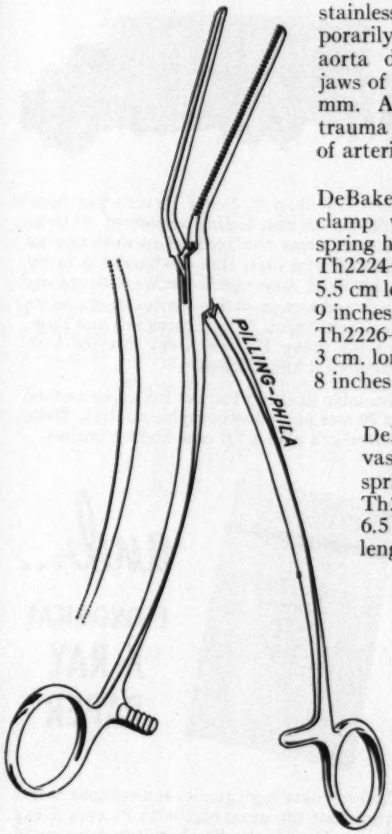

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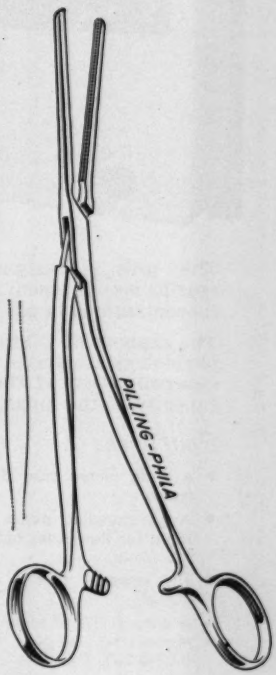

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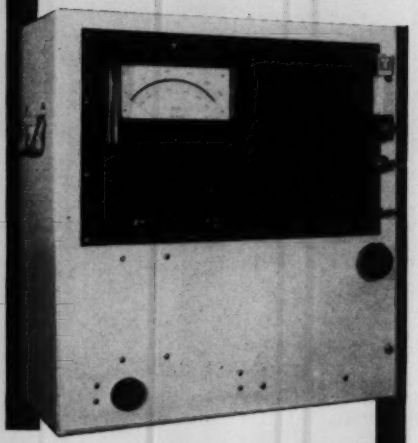

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ERRATUM

In the September, 1960, issue of the REVIEW (volume 82, page 304), in the article, "Studies of Bacillary Populations in Experimental Tuberculosis of Mice Treated by Isoniazid," by G. Canetti, F. Grumbach, and J. Grosset, the legend to figure 5 should read the same as the legends to figures 1–4, namely: X——X = 0.1 mg. isoniazid

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THE AMERICAN REVIEW
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Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

VOLUME 82 *December 1960* NUMBER 6

A BACTERIOPHAGE SPECIFIC FOR MYCOBACTERIUM TUBERCULOSIS, VARIETIES HOMINIS AND BOVIS[1, 2, 3]

W. B. REDMOND AND JEROME C. CATER

(Received for publication August 1, 1960)

INTRODUCTION

Although several bacteriophages lytic for human tubercle bacilli have been isolated, it appears that each is lytic for several other strains, especially saprophytic strains. Most of these phages have been isolated from soil enriched with heavy suspensions of saprophytic mycobacteria. Thus, Froman, Will, and Bogen (1) isolated four phages lytic for both pathogenic and nonpathogenic acid-fast bacilli from soil samples to which saprophytic acid-fast bacilli only had been added. However, no phage was found when pathogenic mycobacteria were added as enrichment to the soil. Takeya and Yoshimura (2) enriched soil with *Mycobacterium avium* and saprophytic strains of mycobacteria, and from these soil samples they isolated subsequently four phages active on saprophytic, human, avian, murine and cold-blooded types of acid-fast bacilli. Both attenuated and pathogenic human mycobacteria were attacked by phages isolated by Hikawa (3) from soil containing animal and chicken feces and enriched with saprophytic acid-fast bacilli.

On the other hand, Piguet (4) reported finding in the digestive tract of a mosquito larva a phage which lysed human pathogenic mycobacteria as well as nonpathogenic strains. Attempts made by Hnatko (5) with a large number of phages to induce adaptation to pathogenic strains by culture methods failed to produce phage lytic for human tubercle bacilli. Soil samples enriched with H37Rv over a period of six months failed also to yield phage. In another experiment, Hnatko (6) enriched dung soil with tubercle bacilli for eight months, but found no phage.

It is apparent that the broad spectrum of activity of the currently available phages limits markedly the use of the phage-typing methods on the mycobacteria. Although groups may be differentiated with the present phages (3, 7–9), no method of identifying specific strains has been reported.

Recently, the present writers have isolated a strain of phage which lyses human and bovine strains of tubercle bacilli but does not lyse the unclassified or the saprophytic mycobacteria. This report consists of studies on this phage.

MATERIALS AND METHODS

The phage was isolated from soil following an enrichment procedure similar to that used by Hnatko (6). The soil was obtained from a stockyard through which animals pass to be slaughtered. Four soil samples, 40 gm. in 250-ml. Erlenmeyer flasks, were each enriched by four additions of a concentrated suspension of ATCC #607 in 7 to 10 ml. of nutrient broth at five-day intervals. This was followed by the addition of 14 similar suspensions of *Mycobacterium tuberculosis* strain H37Rv in nutrient broth. Two soil samples were enriched in a similar manner, but with H37Rv only.

The #607 organisms were grown in nutrient broth (Difco) with aeration. The H37Rv bacilli were grown in a Tween®-albumin liquid medium

[1] From the Veterans Administration Hospital, Atlanta 19, Georgia; and the Department of Microbiology, Emory University, Atlanta 22, Georgia.

[2] This investigation was supported, in part, by a research grant No. E-1371 (c) from the Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

[3] The results reported in this paper were presented at the meeting of the Society of American Bacteriologists, Philadelphia, Pennsylvania, May 5, 1960.

TABLE 1

RESULTS OF TESTS ON VARIOUS STRAINS OF MYCOBACTERIA WITH DS6A PHAGE

Strains Tested with DS6A	Number	Phage Activity*
Human strains recently isolated	196	+
Human strains, laboratory	21	+
Isoniazid-resistant	5	+
Streptomycin-resistant	4	+
Cycloserine-resistant	4	+
Bovine and BCG	3	+
Avian	7	−
Vole	1	−
M. fortuitum	2	−
Unclassified	158	−
Saprophytes	12	−

* + = strains lysed; − = strains not lysed.

adapted by Redmond (10) and containing 1,000 γ of ammonium chloride per ml. In each case the organisms were washed several times in nutrient broth before being added to the soil. The flasks containing the soil were incubated at 37°C. Five days after the last addition of bacilli, 15 ml. of nutrient broth were added to each flask and, after thorough mixing, the contents were allowed to settle at room temperature. The supernatant was pipetted off and centrifuged. After two additional centrifugations, the supernatant was passed through an ultrafine sintered glass filter. Aliquots of the filtrates were mixed with test strains of mycobacteria and spread onto appropriate solid medium. For plating of ATCC #607 and other saprophytic strains, nutrient agar plates were used. The soft agar technique as adapted by Bowman (11) was followed.

The contents of the nutrient agar were as follows: nutrient broth (Difco), 8.0 gm.; sodium chloride, 5.0 gm.; glycerol, 16 ml.; calcium chloride, 1 ml. of a 1 M solution; agar, 11.0 gm. per liter. The pH was adjusted to 7.0.

Dubos oleic-acid–albumin agar (12) and Middlebrook 7H9 agar (13), each with slight modifications, were used for testing the phage on H37Rv and other tubercle bacilli, including the unclassified strains. To the 7H9 agar B (Difco) were added 2 ml. of glycerol, 100 ml. of oleic-acid–albumin complex, 2 gm. of glucose, and 1 ml. of catalase[4] per liter.

For the production of antiserum, rabbits were injected intravenously with 5 to 7 ml. of a heavy suspension of phage in nutrient broth three times weekly for five weeks. The suspensions contained 2×10^{11} phage particles per milliliter.

For the electron microscopic studies the phage was cleaned and concentrated by alternate low- and high-speed centrifugations. The specimens were deposited on collodion- or carbon-covered grids and allowed to air dry partially. This method was previously described by Backus and Williams (14) and used by Bowman (11). The specimens were examined in an RCA, EMU-2 electron microscope.

[4] Manufactured as Armalase by Armour and Company.

RESULTS

A phage, designated DS6A, was isolated from one of the soil samples which had been enriched with both ATCC #607 and H37Rv strains. In addition to the DS6A, several phage strains active on #607 were isolated from this flask. Only the DS6A phage lysed the H37Rv strain. It did not lyse the #607 strain. Tests made on the soil sample prior to the addition of H37Rv revealed the presence of several phages, none of which lysed H37Rv. The two soil samples enriched only with H37Rv produced no phage.

More than 300 strains of mycobacteria were tested with phage DS6A. The distribution of these bacterial strains and their susceptibility to DS6A phage are shown in table 1. Only the human and bovine strains of *M. tuberculosis* and derivatives of these strains were lysed by DS6A. The tests have included 196 strains recently isolated from tuberculous patients, each culture having been identified as tubercle bacilli at the hospital in which it was isolated. The cultures were received from ten different hospitals located in nine states.

The avian, vole, *M. fortuitum*, 12 saprophytic, and 158 strains of unclassified mycobacteria were not lysed. Two species of Nocardia, *N. asteroides* and *N. rubra*, were not lysed by the DS6A phage.

Strains of tubercle bacilli made resistant to streptomycin, isoniazid, PAS, or cycloserine showed no change in susceptibility to the phage.

A slight difference in the appearance of plaques produced on the H37Rv strain and on the bovine Ravenel strain is shown in figure 1. Plaques on the bovine strain were more turbid than those on the H37Rv strain. The plaques continued to enlarge over a period of twelve to fourteen days, attaining a maximal diameter of 10 to 12 mm.

After the DS6A phage had been passaged for three weeks on H37Rv, a plaque type of variant was observed. From one of the variant plaques a phage, DS6B, was isolated and has been used along with DS6A in all tests. On H37Rv the plaques of DS6B were more turbid and the margins of the plaques were not as distinct as DS6A plaques. These differences are shown in

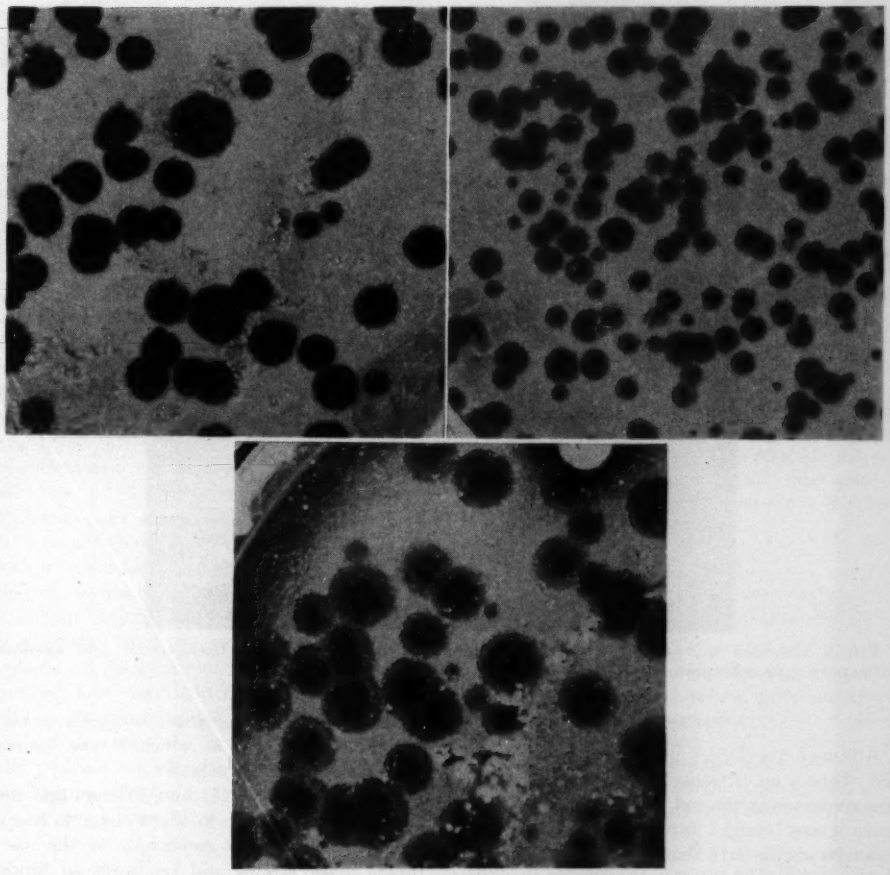

FIG. 1. Plaques illustrating differences in the DS6A and DS6B phages. (Upper left). DS6A phage on strain H37Rv. (Upper right). DS6B phage on strain H37Rv. (Lower). DS6A phage on bovine (Ravenel) strain.

figure 1. Only in 3 recently isolated strains of tubercle bacilli have there been differences in host affinity between the DS6A and DS6B phages. These three strains were lysed by the DS6A but not by the DS6B phage.

The morphology of the DS6A phage is shown on the electron micrograph in figure 2. The head of the phage particle appears almost spherical, with a diameter of approximately 75 to 80 millimicrons. The tail is about 225 millimicrons in length.

Heating at 56°C. for sixty minutes inactivated 75 to 80 per cent of the DS6A phage. At 70°C., the phage was completely inactivated in ten minutes.

Irradiation with ultraviolet for ten minutes inactivated 90 to 95 per cent of the DS6A phage. Irradiation of tubercle bacilli under identical conditions for five minutes killed all bacteria. The dose of ultraviolet was estimated to be 650 ergs per minute.

Antiserum produced against the DS6A phage had the same effect on both the DS6A and the DS6B phages. A dilution of 1:100 of the antiserum inactivated 85 to 90 per cent of the phage in thirty minutes.

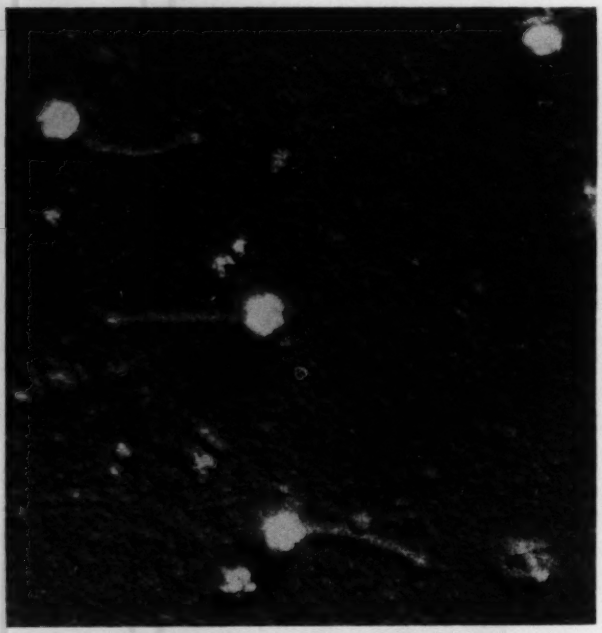

FIG. 2. Electron micrograph of the DS6A phage particles on carbon background and shadowed with platinum. (Magnification, 96,000 ×)

DISCUSSION

Although the DS6A phage was isolated from soil, there is no certainty that this specific phage was contained in the soil, even in small numbers, when it was brought into the laboratory. On the other hand, the fact that tests on the soil prior to the addition of H37Rv showed no phage that lysed the H37Rv strain does not preclude its presence. The importance revolving about the question of the origin of the DS6A phage does not concern so much the origin of this specific phage as it does the method involved in isolating the phage, and whether or not the same method can be used to isolate phages specific for other strains. If a phage not active on H37Rv has been adapted to lyse this strain by being propagated in the presence of H37Rv organisms, it should be possible under appropriate conditions to adapt this or related phage to lyse other strains of mycobacteria. Isolation of another phage, DS2 (15), by the same method lends support to this hypothesis.

Hewitt (16) emphasizes the possibility of adaptations by showing how strains of both phage and bacteria change, resulting in many variant phages, each of which is lytic for some bacteria but not for others.

Although Hnatko (5) and Froman and associates (1) were unable to adapt phage to lyse the H37Rv strain, other conditions or the use of other phage strains will be likely to produce positive results. The results of the present experiments indicate that phage which does not attack certain strains of mycobacteria may be adapted to lyse these strains specifically.

The method used in isolating the DS6A phage and the DS2 phage isolated by Cater and Redmond (15), involving enrichment of a soil sample with the saprophytic strain ATCC #607 followed by enrichment with H37Rv, or by *M. phlei* in the latter case, may offer some basis for a theory of the mechanism of phage adaptation. In both of these instances, phage lytic for #607 but not for either of the other two strains was found in the soil prior to enrichment. The titer of this phage was considerably increased by the addition of #607 to the soil. Under the laboratory conditions used in the isolations, it is

possible—or even likely—that some of the saprophytic organisms may grow and reproduce for a short time. In view of the findings of Waksman (17) and others, the pathogenic tubercle bacilli would be expected to be destroyed in a relatively short time by various agents found in the soil. Deoxyribonucleic acid conceivably may be liberated from these bacilli with little change. Although the free phage present in the soil would not be affected by the deoxyribonucleic acid, there are still susceptible saprophytic bacilli remaining and probably multiplying in the soil. A few of these may be able to absorb deoxyribonucleic acid from the tubercle bacilli and, if one such bacillus is infected and lysed by one of the phage particles present, the resulting phage particles may conceivably be influenced by the absorbed deoxyribonucleic acid and take on some of the characteristics of the tubercle bacilli. Such an adaptation may lead to a phage particle that would be active on the tubercle bacilli.

Other mechanisms may become apparent following additional isolations of phage specific for certain strains, especially if other methods are successful. With the present interest in and emphasis on deoxyribonucleic acid, the theory presented here may help to explain the results obtained by the writers. The isolation of phage specific for strains of the unclassified groups would make possible considerable progress toward the phage typing of the mycobacteria. Such isolations are being attempted.

Summary

A phage, designated DS6A, was isolated from soil from a stockyard following enrichment of the soil samples with ATCC #607 and H37Rv. Phage DS6A and a variant, DS6B, are lytic for pathogenic human and bovine tubercle bacilli but not for saprophytic or unclassified strains of mycobacteria.

More than 300 strains of mycobacteria, including 196 pathogenic strains recently isolated from tuberculous patients, have been tested with the two DS6 phages. Antiserum has been produced in rabbits and is 95 per cent inhibitory for the phage in 1:100 dilution. Heating at 56°C. for sixty minutes inactivated 75 to 80 per cent of the phage. Slightly less than 90 per cent of the phage was inactivated in ten minutes by a dose of ultraviolet that killed all tubercle bacilli in five minutes.

Sumario

Un Bacteriófago Específico para el Mycobacterium Tuberculosis variedades hominis y bovis

Del suelo de un corral de ganado, se aisló un bacteriófago, denominado DS6A, consecutivamente al enriquecimiento de muestras del suelo con ATCC #607 y H37Rv. El fago DS6A y una variante, DS6B, son líticos para bacilos tuberculosos humanos y bovinos patógenos, pero no para cepas saprofitas o inclasificadas de micobacterias.

Con los dos bacteriófagos DS6 se han comprobado más de 300 cepas de micobacterias, comprendiendo 196 cepas patógenas recién aisladas de tuberculosos. Se ha producido antisuero en conejos, mostrando un 95 por ciento de inhibición para el fago a una dilución de 1:100. La calefacción a 56°C. durante sesenta minutos inactivó de 75 a 80 por ciento del fago. Un poco menos de 90 por ciento del fago fué inactivado en diez minutos por una dosis de rayos ultravioletas que mató a todos los bacilos tuberculosos en cinco minutos.

Resume

Un bactériophage spécifique des variétés de Mycobactérium tuberculosis humain et bovin

Un bactériophage, nommé DS6A, a été isolé à partir du sol d'une cour à bétail, après enrichissement des échantillons du sol avec ATCC # 607 et H37Rv. Le bactériophage DS6A et une de ses variétés, DS6B, sont lytiques pour les bacilles tuberculeux humains et bovins pathogènes, mais pas pour des souches de mycobactéries saprophytes ou non classées.

Plus de 300 souches de mycobactéries, y compris 196 souches pathogènes furent récemment isolées chez des malades tuberculeux, et ont été examinées avec les deux bactériophages DS6. Un antisérum a été produit chez des lapins, et il inhibe à 95% le bactériophage dilué à 1:100. Chauffer à 56°C. pendant soixante minutes inactiva de 75 à 80% le bactériophage. Un peu moins de 90% de bactériophage fut inactivé en dix minutes par une dose d'ultra-violets qui tua tous les bacilles tuberculeux en cinq minutes.

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THE EFFECT OF CHANGE IN BODY POSITION ON LUNG VOLUMES AND INTRAPULMONARY GAS MIXING IN PATIENTS WITH OBESITY, HEART FAILURE, AND EMPHYSEMA[1, 2]

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INTRODUCTION

Postural alterations in certain subdivisions of the lung volume and in intrapulmonary gas mixing have been previously demonstrated in normal subjects (1–6). The functional residual capacity becomes progressively smaller as the subject changes from the erect to the seated and finally to the Trendelenburg position. This alteration occurs at the expense of the expiratory reserve volume, with little alteration of the residual volume (5). The decrease in functional residual capacity is believed to be secondary to the progressive elevation of the diaphragm by the abdominal viscera during recumbency. In addition, most normal seated subjects demonstrate a slowly ventilated space as measured by the clearance of nitrogen or helium from the lung. Although this space is not significantly different in the erect or seated position, it becomes smaller and more slowly ventilated when the subject is recumbent (5).

The purpose of this study is to extend the observations of the postural effects on the subdivisions of lung volume and intrapulmonary gas mixing to patients with obesity, heart failure, and pulmonary emphysema. Dyspnea is a common and serious complaint in all three conditions. The severity of this symptom may be varied by changes in body position. It is believed that a comparison of the effects of alteration in body position on subdivisions of lung volume and intrapulmonary gas mixing in these three conditions may help to elucidate the factors involved in the respiratory symptoms and findings which these patients demonstrate.

[1] From the Department of Medicine, Duke University Medical Center, Durham, North Carolina.

[2] Supported, in part, by grants from the American Heart Association and the Edgecombe Nash County Heart Association (North Carolina Heart Association).

METHODS

Observations were made on 35 male patients. Thirteen of the group were obese, with an average weight of 130 kg. and an average age of forty-three years; 6 patients had heart failure and an average age of forty-five years; 16 patients with an average age of fifty-one years had pulmonary emphysema. The obese patients were asymptomatic except for the complaint of dyspnea. Measurements of the lung volume and its subdivisions and the most slowly ventilated space were made with the patient seated and recumbent. The patients with emphysema were also studied in the Trendelenburg position. The results from this study were compared with those obtained from 9 normal subjects previously reported by this laboratory (5).

The vital capacity and its subdivisions were measured in the conventional fashion with a 13-liter Collins spirometer. The functional residual capacity and the volume and rate of ventilation of the pulmonary slow space were measured by an open-circuit helium method (7). By this method, the patient breathes a mixture of 50 per cent helium and 50 per cent oxygen for fifteen minutes in order to achieve a nearly uniform intrapulmonary helium concentration. At the end of a normal expiration, the patient is switched to 100 per cent oxygen, and the expired gas is collected for volume and helium concentration. The helium concentration in the expired gas is followed continuously with a sensitive katharometer between levels of 3.30 and 0.05 per cent. The functional residual capacity is calculated from the total quantity of helium removed during the washout period and the mean intrapulmonary concentration of helium at the start of the washout.

For measurement of the slow space, the helium concentration in the expired air is plotted against time on a semi-logarithmic graph. The terminal part of this curve, which is produced by the helium excretion from the slow space, approximates a straight line. From the slope of this line, the point at which it intersects the ordinate at zero time, and the minute ventilation rate of the patient, it is possible to estimate the ventilation rate and volume of the slow space. In this study, the expressions "k_s" and "f" are used to characterize the slow space. "K_s" is the "turnover rate" of the slow space or the ratio of its minute ventilation to its volume. The relative size of the slow space is expressed as "f" or the ratio between the volume

TABLE 1

POSTURAL EFFECTS ON LUNG VOLUME AND INTRAPULMONARY GAS MIXING

Type of Subject	Number	Age	Position	Vital Capacity	Inspiratory Capacity	Expiratory Reserve	Functional Residual Capacity	Residual Volume	Total Capacity	Turnover Rate of Slow Space (k_s)	Relative Volume of Slow Space (f)
Normal	9	31	Seated	4.96 ± 0.55	3.18 ± 0.50	1.80 ± 0.32	3.30 ± 0.45	1.40 ± 0.52	6.36 ± 0.59	0.93 ± 0.48	0.37
			Recumbent	4.64 ± 0.43*	3.70 ± 0.56*	0.94 ± 0.21*	2.62 ± 0.60*	1.58 ± 0.60	6.22 ± 0.65	0.45 ± 0.32	0.21
Obesity	13	43	Seated	3.11 ± 0.79†	2.43 ± 0.47†	0.67 ± 0.43†	2.30 ± 0.65†	1.63 ± 0.45	4.74 ± 1.03†	0.50 ± 0.21†	0.32 ± 0.21
			Recumbent	2.87 ± 0.89†	2.59 ± 0.76†	0.29 ± 0.20†	1.95 ± 0.35†	1.66 ± 0.39	4.54 ± 0.87†	0.31 ± 0.09*	0.26 ± 0.14‡
Heart failure	6	45	Seated	1.38 ± 0.39†	1.05 ± 0.22†	0.35 ± 0.22†	1.76 ± 0.27†	1.40 ± 0.30	2.78 ± 0.54†	0.78 ± 0.52	0.26 ± 0.08
			Recumbent	1.38 ± 0.41†	1.20 ± 0.41†	0.22 ± 0.09†	1.49 ± 0.35†	1.16 ± 0.28	2.54 ± 0.53†	0.87 ± 0.98	0.33 ± 0.17
Obstructive emphysema	16	51	Seated	2.51 ± 0.58†	1.65 ± 0.46	0.86 ± 0.34†	5.11 ± 0.99†	4.22 ± 1.06†	6.76 ± 1.06	0.24 ± 0.10†	0.72 ± 0.14†
	12		Recumbent	2.34 ± 0.64†	1.58 ± 0.48†	0.76 ± 0.33	4.92 ± 1.36†	4.15 ± 1.24†	6.50 ± 1.35	0.20 ± 0.11†	0.64 ± 0.20†
	11		Trendelenburg	2.44 ± 0.67	1.67 ± 0.66	0.77 ± 0.24	4.82 ± 1.09	4.02 ± 1.11	6.46 ± 1.01	0.22 ± 0.09	0.73 ± 0.14

Measurements are all in liters

* Significantly different from the value in the seated position ($P < 0.01$)

† Significantly different from the value in the normal group ($P < 0.01$)

‡ Significantly different from the value in the seated position ($P < 0.02$)

of the slow space and the functional residual capacity.

RESULTS

The results of this study are summarized in table 1 and figure 1. It has been noted previously that normal subjects decrease their vital capacity, expiratory reserve, and functional residual capacity when they assume the recumbent position (5). This is due to a decrease in expiratory reserve. Despite a decrease in the recumbent vital capacity, the inspiratory capacity increases, indicating a shift in the resting mid-position of the lung. Most normal people have a slowly ventilated space of the lung which becomes smaller and more slowly ventilated in the recumbent position.

The seated obese patients demonstrated measurable decreases in all pulmonary subdivisions except the residual volume as compared with the values for normal subjects. Mean values for vital capacity and total lung volume in the obese group were 3.11 liters and 4.74 liters, respectively, compared with 4.96 liters and 6.36 liters in the normal group. The slowly ventilated space of the seated obese patient was similar in size and ventilation rate to that of the normal recumbent subject. The most striking alteration

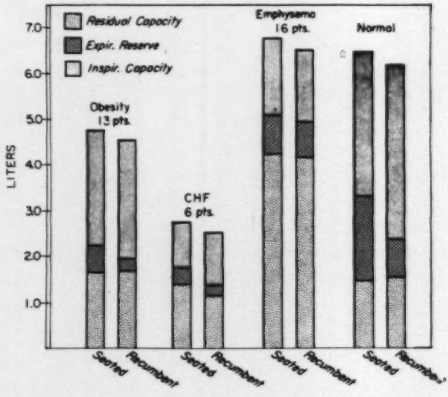

FIG. 1. Postural effects on lung volume and subdivisions compared for normal subjects and for patients with obesity, heart failure, and emphysema.

which occurred in the lung subdivisions on change to the recumbent position was a fall of the expiratory reserve to a mean value of 290 cc. for the group of 13 patients, compared with the normal recumbent mean value of 940 cc. During recumbency, the obese patients as a group showed a further change in the slowly ventilated

space from a mean k_s of 0.50 to 0.31 and with a mean change in f from 0.32 to 0.26.

Patients with heart failure, like the obese patients, demonstrated a significant decrease in all subdivisions of the lung volume except the residual volume when measurements were made in the seated position. The slowly ventilated space in the seated position was similar in size and ventilation rate to that of normal subjects. However, unlike normal or obese subjects, the patients with heart failure did not demonstrate a change in the slowly ventilated space when in the recumbent position.

The decreased vital capacity, enlarged functional residual capacity, and large slowly ventilated space which were observed in patients with pulmonary emphysema were unaffected by body position. Although several emphysematous patients noted subjective improvement in breathing in the Trendelenburg position, this could not be correlated with a change in the size or ventilation rate of the slow space.

Discussion

The inequality of ventilation of the lungs in normal subjects in the recumbent and Trendelenburg positions has been attributed to a progressive elevation of the diaphragm due to pressure from the abdominal viscera or a decrease in the resting tone of the diaphragm (5). Despite the presence of a slowly ventilated space, the arterial oxygen saturation is normally maintained at levels of 94 to 96 per cent. This suggests that, under normal circumstances, the perfusion distribution or rate is altered in the region of the slowly ventilated space. Actually, a physiologic shunting of blood away from the slowly ventilated area may occur and so prevent arterial unsaturation, even in the presence of localized hypoventilation. Apparently, both oxygen and carbon dioxide tensions at the level of the alveolus can alter pulmonary vascular resistance (8–10).

Recently, the adverse effects of obesity on cardiac and pulmonary function have been emphasized (11–13). A cardiopulmonary syndrome consisting of somnolence, periodic breathing, hypoxemia, hypercapnia, polycythemia, and heart failure has been described in association with marked obesity in some patients. The alveolar hypoventilation reported in this syndrome has been ascribed to a compromise in ventilatory effort which allows carbon dioxide tension to rise rather precipitously. It has been suggested that the effort necessary to keep the carbon dioxide tension normal is not exerted because of either unresponsiveness of the medullary respiratory center to this stimulus or the mechanical restriction of pulmonary ventilation. Whether the development of alveolar ventilation is the result of increased work of breathing and a compromise by the patient because of the cost of breathing, or whether central nervous system depression is a primary etiologic feature of the syndrome, has not been settled completely. However, it should be noted that early in the illness, or as the patient begins to lose weight and recover, marked changes in arterial oxygen saturation are observed with periodic breathing or with momentary breath-holding (11). These rapid changes in arterial oxygen saturation are believed to be the result of a combination of increased oxygen consumption by the body as a whole and a decrease in the functional residual capacity or the volume of air contained in the lung at the end of a normal expiration. The further decrease in functional residual capacity observed in the obese patient during recumbency would tend to aggravate this process.

In these studies, the seated obese patient has been found to have a slowly ventilated space in the lungs which is similar in size and ventilation rate to that observed in the normal recumbent individual. Bedell, Wilson, and Seebohm (14) have also observed a prolongation of the nitrogen clearance in patients with obesity and associated lung disease or myxedema. Those writers suggested that arterial hypoxemia is observed in some patients with obesity without lung disease because of a disturbance in ventilation and perfusion equality within the lungs. From observations in obese patients without evidence of the obesity cardiopulmonary syndrome, it is evident that the slow space within the lung, although it is slightly decreased in size, is more slowly ventilated when the patient is recumbent. This may become particularly significant in obese patients who are somnolent or in obese persons during recumbent sleep with depression of ventilation. Under these circumstances, perfusion of the areas representing the slowly ventilated space would result in arterial oxygen unsaturation and, in effect, would represent a shunt from the right to the left side of the heart,

as observed in some patients with the obesity cardiopulmonary syndrome (15).

The marked decrease in all subdivisions of the lung volume except the residual volume in heart failure has been reported in the past (16, 17). With change to the recumbent position, no further significant decreases in any subdivisions were noted. Although the changes in lung subdivisions in the obese patient and in the individual with heart failure were of similar magnitude, most of the patients with heart failure had measurable slowly ventilated spaces similar to that of normal subjects. Unlike normal or obese subjects, there was no change with recumbency. The explanation for this difference is not clear. However, it has been suggested that the lungs of patients in heart failure are more rigid and, because of the decrease in compliance, move more uniformly, therefore abolishing a slowly ventilated space (18).

The marked increase in the residual volume and the decrease in the vital capacity, inspiratory capacity, and expiratory reserve observed in the patients with obstructive emphysema are well recognized. The large and slowly ventilated space has also been previously reported (19, 20). The lung subdivisions and the large, slowly ventilated space were not significantly altered in emphysematous patients with a change from the seated to the recumbent and Trendelenburg positions. The subjective improvement in breathing and blood gases reported to occur in emphysematous patients in the Trendelenburg position cannot be explained on the basis of measurable alteration of the lung subdivisions or improvement in intrapulmonary gas mixing.

Summary

The lung volume, its subdivisions, and intrapulmonary gas mixing were compared in the seated and recumbent positions in normal subjects and in groups of patients with obesity, heart failure, and pulmonary emphysema. The obese patients had significant decreases in all lung subdivisions except the residual volume. The expiratory reserve, in particular, became extremely small in the recumbent position. The slowly ventilated space in the seated position was comparable to that observed in the recumbent normal individual. Moreover, when the obese patient was recumbent, the space became even more slowly ventilated. It is suggested that ventilation-perfusion inequalities, particularly those involving slowly ventilated areas, may contribute to the arterial hypoxemia observed in some patients with obesity.

By contrast, patients with congestive heart failure who also had decrease in the subdivisions of the lung except for residual volume did not demonstrate a space which was very slowly ventilated and showed no change with recumbency. Patients with pulmonary emphysema had slowly ventilated spaces which were larger than those observed in the obese patients, but they were ventilated at roughly the same rate as those of the recumbent obese patient.

Sumario

El Efecto de los Cambios en la Postura del Cuerpo sobre los Volúmenes Pulmonares y la Mezcla Intrapulmonar de Gases en los Enfermos que Padecen de Obesidad, Insuficiencia Cardíaca y Enfisema

Se compararon el volumen pulmonar, sus subdivisiones y la mezcla intrapulmonar de gases en las posiciones sedente y recostada en sujetos normales y en grupos de enfermos de obesidad, insuficiencia cardíaca y enfisema pulmonar. Los obesos mostraron disminuciones importantes en todas las subdivisiones pulmonares, exceptuando el volumen residual. En particular, la reserva espiratoria se volvió sumamente baja en la posición recostada. El espacio que se ventiló lentamente en la posición sedente era comparable al observado en el individuo normal recostado. Además, cuando el obeso estaba recostado, el espacio se ventilaba aun con mayor lentitud. Apúntase que las desigualdades en la ventilación-perfusión, y en particular las que afectan zonas de ventilación lenta, pueden contribuir a la hipoxemia arterial observada en algunos obesos.

En contraposición, los enfermos de insuficiencia cardíaca congestiva, que también mostraron disminución en las subdivisiones del pulmón, exceptuando el volumen residual, no revelaron espacios de ventilación muy lenta ni acusaron cambio alguno al tomar la posición recostada. Los enfermos de enfisema pulmonar tenían espacios de ventilación lenta, mayores que los observados en los obesos, pero que se ventilaron más o menos con la misma rapidez que los de los obesos recostados.

Resume

Effet du changement de position du corps sur les volumes pulmonaires et le mélange intrapulmonaire de gaz chez des malades obèses, en défaillance cardiaque et emphysèmateux

Les auteurs ont comparé le volume pulmonaire, ses subdivisions et le mélange intrapulmonaire de gaz dans les positions assise et couchée, chez des

sujets normaux et chez des groupes de malades obèses, en défaillance cardiaque et avec emphysème pulmonaire. Les malades obèses eurent une diminution nette de toutes les subdivisions pulmonaires, excepté le volume résiduel. La réserve expiratoire, en particulier, devint très petite dans la position couchée. L'espace lentement ventilé dans la position assise put être comparé à celui observé chez le sujet normal couché. En plus, quand le malade obèse était couché, l'espace devint encore plus lentement ventilé. Ce qui fait penser que les inégalités de ventilation et de perfusion, particulièrement celles qui intéressent les régions lentement ventilées, peuvent contribuer à l'hypoxémie artérielle observée chez certains malades obèses.

Au contraire, des malades avec défaillance cardiaque, qui avaient aussi une diminution des subdivisions pulmonaires excepté le volume résiduel, ne montrèrent pas d'espaces ventilés lentement et ne montrèrent pas de changement dans la position couchée. Des malades avec emphysème pulmonaire avaient des espaces lentement ventilés qui étaient plus larges que ceux observés chez les malades obèses, mais qui étaient ventilés à peu près à la même vitesse que celle des malades obèses couchés.

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PATHOLOGY OF PULMONARY EMPHYSEMA[1]

Methods of Study

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Introduction

With growing clinical interest in chronic bronchitis and emphysema there is an increasing pressure on the pathologist for accurate descriptions of emphysema in autopsy material. In a preliminary article (1), the writer described a method for pressure-fixing lungs, and further observations and illustrations were published later (2, 3).

The apparatus described in the first article was effective but primitive, and the first objective of the present article is to describe a new apparatus which fixes several lungs at one time and requires less attention. A continuous pressure of 25 to 30 cm. of aqueous formalin is applied via the bronchi for seventy-two hours. The second objective is to give reasons for adopting that particular pressure. The third objective is to describe a rapid syphon method for measuring the volumes of the distended lungs and to give evidence to suggest that the volumes (hence, the air-spaces) are comparable approximately to full inspiration in life. Finally, some other methods for diagnosing emphysema are discussed.

Methods

Pressure-Fixation

Mucus is sucked from the segmental bronchi by means of a cannula attached to a filter pump. After this a larger cannula, made by mounting a rubber bung broad end distally on glass tubing, is tied firmly in the main bronchus and attached to the pressure-fixation apparatus. Cuts and tears are sutured before inflation.

The object of the apparatus is to maintain a constant head of pressure of 25 to 30 cm. unaffected by slow leaks from the blood vessels and pleura. Leaks from a number of lungs would soon empty a large container, and continuous pressure is maintained by using a centrifugal pump to return formalin to the upper container.

The improved apparatus is shown diagrammatically in figure 1. The lower container *A* is two-thirds filled with a 1:4 mixture of 40 per cent formalin:water. The centrifugal pump *P*,[2] protected by the filter *F*, is used to raise the formalin to the upper container *B*, the output being controlled by the tap *C*. The fixative accumulates in the upper container and overflows via the wide-bore tube *D* back into the lower container. The height of the upper container is adjusted to give a distance of 25 to 30 cm. between the fluid levels in each container. The manifold is supplied from the upper container by the tube *E* which, in this case, has been sealed into the center of tube *D* but does not communicate with it. Six outlets from the manifold are controlled by screw clips on rubber tubing, and six lungs or more can be pressure-fixed at one time. The manometer *M* serves to check that outlets not in use are not left open.

Occasional adjustment of tap *C* ensures that formalin overflows into pipe *D* without overreaching it. Fixative is added to replace loss in removed specimens and by evaporation. In view of the strength of formalin employed, the apparatus should be kept in a fume cupboard.

Measuring Lung Volume

The lung is submerged in a tank of water and the overflow is measured. The tank is 30 cm. long, 25 cm. wide, and 28 cm. deep. A metal tube with a 6-mm. bore is let into the wall 6 cm. from one end of the tank, 3 cm. from the top. On the inside it bends down vertically and ends 7 cm. from the top and 1 cm. from the side. On the outside, rubber tubing leads down to a container below the level of the tank. The tank is filled with water and the syphon drains it quickly to a sharp end-point. The lung is completely submerged and the syphon drains off its volume of water, again quickly reaching the sharp end-point. If the syphon does not start spontaneously, the rubber tubing is pumped with the fingers to start it.

Lung volumes of about 3 liters are common, but abnormally large volumes of more than 5 liters have been encountered (*see* Discussion). The end-point is reached by the syphon far quicker than by an ordinary overflow. The whole procedure takes only a few minutes.

[1] From the Department of Morbid Anatomy, Postgraduate Medical School, Hammersmith Hospital, Ducane Road, London, W.12, England.

[2] Motorized centrifugal suds pump, type C.S. 455, 4 to 6 gallons per minute, obtained from Buck and Hickman, 2 Whitechapel Road, London, E.1, England.

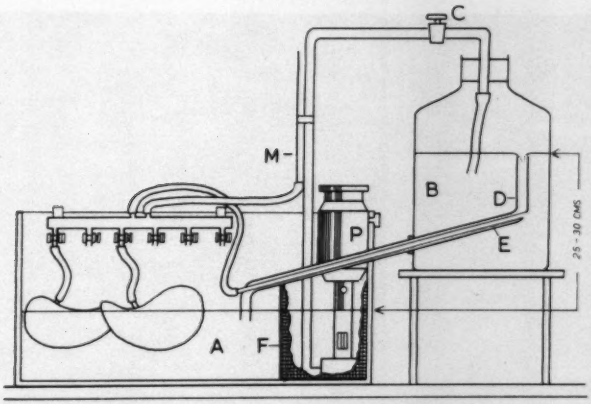

FIG. 1. Pressure-fixation apparatus for applying a continuous pressure of 25 to 30 cm. of aqueous formalin to the main bronchus. The pump *P* raises formalin to the upper container *B* where it overflows down the pipe *D*. The manifold is supplied by the tube *E*, and six lungs (or more) can be fixed at one time. Fixation takes seventy-two hours.

Slicing the Lung

A number of abnormalities which are obvious on external examination of the lung, e.g., bulla on the medial aspect of the upper lobe, are not always sampled in the anteroposterior slice. It has been found useful to draw, briefly, the medial and lateral aspects of the lung, noting pigmentation, adhesions, shrinkage, or enlargement of lobes, and any other features.

Slices are cut on a board fitted down two sides with rails 0.8 cm. high. These support the blade of a 35-cm. ham knife (4). Anteroposterior slices are taken from the lateral half of the lung, leaving the medial half for dissection of bronchi and vessels.

Barium-Sulfate Impregnation

Barium-sulfate impregnation has been devised to reduce the natural translucency of air-space walls. It can be used on unfixed lungs in urgent circumstances, although a short period (thirty minutes) of pressure fixation greatly improves the results.

The selected lung slice is submerged in a flat dish of aqueous barium nitrate (approximately 75 gm. in 1 liter of water). All parts of the slice are pressed with the finger-tips to encourage penetration, and after one minute it is squeezed out lightly and transferred, face down, to a tray of aqueous sodium sulfate (approximately 100 gm. in one liter of water) for one minute. The lung becomes impregnated by a fine white precipitate of barium sulfate. Overimpregnation is to be avoided, but occasionally the procedure can be repeated once with advantage, especially when the solutions are used repeatedly.

The lung slice is examined in water. It becomes pale and opaque with this treatment, but dust pigment shows clearly through the precipitate. Centrilobular emphysema is often pigmented and becomes more distinct against a whitened background. Diffuse (panacinar) emphysema may be missed without impregnation. The diameters of respiratory bronchioles (only measured when seen end on) and alveoli can be measured quickly by the dissecting microscope with graduated eyepiece, and abnormal dilatation (figure 2) or destruction (figure 3) observed.

Storing Lung Slices

After barium-sulfate impregnation, lung slices usually sink in water (when air bubbles have been squeezed out). They are stored in buckets of 5 per cent formalin, resting lightly one on another and not folded. They crease easily and must be stored flat, not heavily compressed.

Incidentally, the tendency for the slices to sink in water after impregnation is most useful. Slices in trays usually lie below the surface of the water, and direct observation and photography are facilitated.

DISCUSSION

The principal reason for pressure fixation is to prevent collapse and irregular shrinkage of the lung while it floats in the fixative. Volume measurements made at intervals during fixation and for some months afterward remained remarkably constant, although some shrinkage has been found in lungs stored for a year or more.

Another reason for pressure fixation is to fix air spaces in a state comparable to that in life. The pressure of 25 to 30 cm. was adopted on the

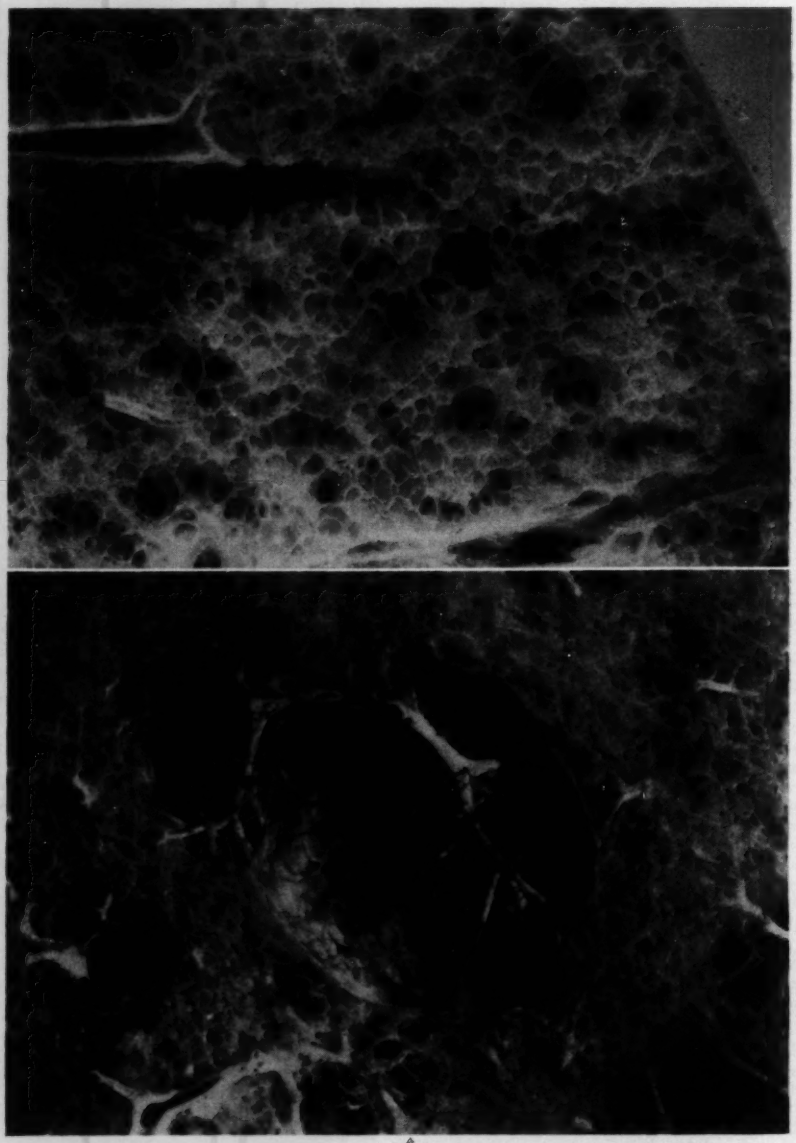

FIG. 2. Mild diffuse (panacinar, panlobular) emphysema in which respiratory bronchioles are abnormally dilated. There is no destruction of air spaces. (× 11, barium-sulfate–impregnated slice)

FIG. 3. Centrilobular emphysema. Bare pulmonary arteries cross the lesions, indicating destruction of respiratory tissue. There is some thinning of the intervening air-space walls, indicating accompanying moderate diffuse (panacinar, panlobular) emphysema. (× 7, barium-sulfate–impregnated slice)

following account. When the chest is opened at autopsy, normal lungs recoil to produce an intratracheal pressure of 2.8 to 6.2 cm. of water (5). Surface alveoli can be filled with air at a pressure of 10 cm. of water (5). The latter pressure is sufficient to fill the normal lung, but the bronchi commonly contain mucus, so the higher pressure 25 to 30 cm. was adopted to overcome minor grades of obstruction. In addition, this higher pressure was required to overcome the lungs' tendency to flatten on the surface of the fixative. Raising the pressure from 30 to 120 cm. increased the volume by only 7 per cent, indicating that, while higher pressures did not alter the volume greatly, they were not necessary for adequate filling to take place. At pressures in the region of 150 cm., rupture of the lung sometimes occurred; there was a sudden elevation of the pleura over the convex medial surface of the lower lobe.

There is a fairly sharp end-point to expansion of the lung with formalin. Once the fixative runs into the uppermost parts of the specimen, the size does not visibly increase very much. Most of the recoil pressure has been overcome merely by the height of fluid within the specimen. Expansion resembles a paper bag rather than a rubber balloon. The limit to expansion is set presumably by reticulin, collagen, and stretched elastic fibers tethered to stretched bronchi and vessels. There is no bulging of concave anatomic depressions.

Since increasing the pressure increases the volume slightly (7 per cent from 30 to 120 mm.), it is wiser to apply the same pressure to each lung if their sizes and air spaces are to be compared. However, the fairly sharp end-point to filling at 25 to 30 cm. did suggest that the lung volume may then approximate that at full inspiration in life. Corresponding retrospective roentgenograms were kindly examined by Dr. J. W. Laws by the method of Barnhard and co-workers (6) to determine the roentgenographic total lung capacity. By omitting to subtract the volume allowed for lung parenchyma, he estimated the total lung volume. By assuming the left lung to be four-ninths of the total (a proportion based on the weights of normal lungs at autopsy), he estimated the approximate volume of each lung.

The results in table 1 show approximate agreement in *Cases 1, 3, 5* and *6*, with differences up to 12 per cent. At first sight the results in *Cases 2* and *4* appear to show gross discrepancy; but the left hemithorax of *Case 2* was relatively enlarged, and in *Case 4* it was abnormally small. When the volumes of the lungs were calculated separately but directly from the roentgenograms, the difference was reduced to 9 per cent in *Case 2* (1,830 ml.) and to 8 per cent in *Case 4* (3,410 ml.).

TABLE 1

A Comparison of the Volumes of Six Lungs Measured after Pressure Fixation and in Corresponding Roentgenograms

Lung	Volume of Pressure-fixed Specimen	Roentgenographic Lung Volume	Difference as Percentage of Roentgenographic Lung Volume
1. Left	2,840	3,170	7
2. Left	2,000	1,560	(28)*
3. Right	4,075	3,640	12
4. Left	3,140	3,820	(17)†
5. Left	3,710	3,670	1
6. Left	3,040	2,820	8

* Left hemithorax relatively enlarged.
† Left hemithorax abnormally small.

It remains to be seen which method gives the truer result, but the values are close enough to support the contention that pressure-fixed lungs (25 to 30 cm.) are approximately comparable in size to full inspiration in life. Izukawa (7), working in this laboratory, found the volume of the left lung in males, pressure-fixed at 25 to 30 cm., to be in the range of 2,200 to 3,500 ml. after the fourth decade of life. Occasionally values of more then 5 liters have been seen in emphysema.

The closeness to the natural size is further exemplified in figure 4, in which the large anterior emphysematous bulla measured 5 cm. in diameter in the roentgenogram in life and in the specimen. (The bulla was filled with agar for cutting and mounting.)

In the chronic bronchitic lung, pressure-fixed, the bronchi are often abnormally wide and may be confused with bronchiectasis. Strictly speaking, any dilatation should be termed bronchiectasis, but at present it is probably better to confine the term to bronchi which actually increase in diameter as they are traced peripherally. Pressure fixation is useful for measuring bronchial diameters.

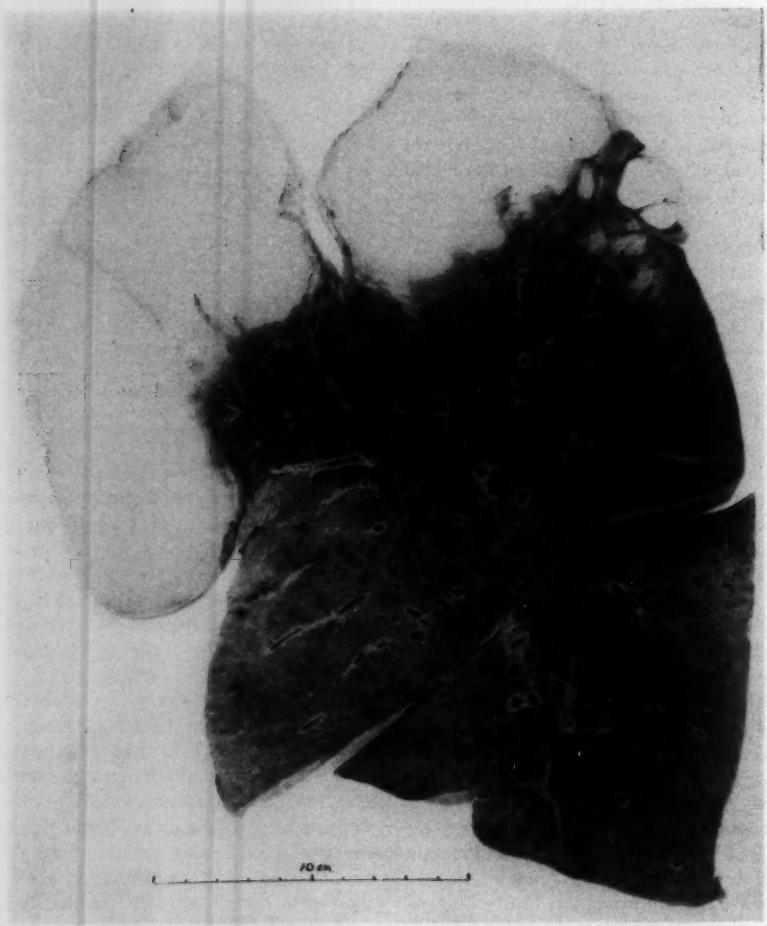

FIG. 4. Large bullae (irregular emphysema) arising superficially from the right upper lobe. The anterior bulla measured 5 cm. in the roentgenogram in life and in this pressure-fixed specimen. The rest of the upper lobe is emphysematous and dust pigmented. There are a few pigmented foci of centrilobular emphysema in the other lobes. (Barium-sulfate–impregnated slice; bullae filled with agar)

Occasionally a lung fails to distend at the pressure of 25 to 30 cm. of fixative. The pressure required to initiate filllng should be recorded, since it seems to be a method for estimating roughly the degree of respiratory obstruction at the time of death. One lung from a case of status asthmaticus failed to expand until a pressure of 180 cm. was applied. In chronic bronchitis, individual segments are occasionally blocked. Once the lung is filled at a higher pressure, it can be fixed on the apparatus at the standard pressure. The majority of lungs fill at 25 to 30 cm. in less than five minutes.

Barium-sulfate impregnation is especially valuable in detecting thinning and absence of alveolar walls, as in diffuse (panacinar) emphysema. Photography is facilitated. It should be appreciated that this procedure is designed essentially to render fine air spaces more easily visible and measurable *to detect emphysema*. The staining methods of Blumenthal and Boren (8), Tobin (9), the demonstration of elastic, as by

Carton and associates (10), and other procedures will yield additional information. The use of arterial injections in combination with barium-sulfate impregnation was demonstrated previously (1).

Older methods of fixation have been described by Miller (11). A common scheme at the present time is to run formalin down the main bronchus from a container raised about 150 cm. above it The end-point varies with the operator, usually stated as "avoiding overinflation." The lung is floated in a bucket of fixative for some time and, as it flattens out on the surface, the volume may fall by one-third or more. Barium-sulfate–impregnated slices of such preparations show shallow air spaces in various degrees of collapse and distortion.

Hartroft and Macklin (12) were aware of the importance of fixing methods when they measured alveoli. They allowed formalin to run down the trachea a little at a time, and the lungs, immersed in a bath of 3 per cent formalin, were gently moved about to allow the air bubbles to escape. When bubbles no longer emerged, formalin at a pressure of 75 cm. of water was injected into the bronchi until the length of the lung corresponded to the intrathoracic measurement (cupola to lower border of eleventh rib at the costovertebral junction). The lungs were clamped off, and the length checked from time to time for three weeks or more. Alveoli were measured histologically. In a woman of thirty-one they were 190.13 microns in average diameter, similar to the sizes less finely measured in the present preparations (0.15 to 0.2 mm.). It seems likely that the specimens in that study were not far from full inflation. Air-bubble artefact does not occur with pressure fixation so it is not necessary to run the formalin in slowly. In fact, this may be a disadvantage, allowing mucus to become partly fixed and block bronchi before the main pressure is applied.

Other investigators fix the lungs *in situ* by perfusion via the circulation. Histologic preservation is good, but patchy incomplete expansion of basal segments, a common finding at autopsy, is perpetuated by this method.

For many years lungs have been distended with air and dried for dissection, e.g., Waters in 1862 (13). Tobin (9) applied compressed air at a pressure of 5 to 50 mm. of mercury, depending on the elasticity of the particular lung. He described a variety of procedures for examining structural details. Oderr, Pizzolato, and Ziskind (14, 15) have also used inflation drying for the diagnosis of emphysema, with various transillumination and microradiographic techniques. Blumenthal and Boren (8) added formalin vapor to the pressurized air before drying out the lung, cutting it with a band-saw and staining the slices. Duguid (16) has also been fixing lungs recently with formalin vapor to reduce debris in air spaces before using an electronic scanning machine to determine respiratory surface. The formalin vapor technique is an interesting development but may not be reliable for studying air-space size. It is not yet certain whether the volumes of such specimens and, thus, the sizes of air spaces are preserved in a state comparable with that in life. The intrapulmonary pressure—hence the final volume—would be more seriously reduced by air escaping from punctures in the pleura than by aqueous formalin.

The method of Gough and Wentworth (17) for mounting giant sections on paper has an established place in recording pneumoconiosis and has been used for diagnosing emphysema by Wyatt (18) and Leopold and Gough (19, 3); a three-dimensional approach is achieved by using serial sections (20).

McLean (21) took successful photographs of cut surfaces of lungs by attention to lighting technique. Barium-sulfate impregnation now allows higher magnifications to be studied and photographed (1, 2, 3).

Broad recommendations for methods of study of emphysema have been made at the two symposia on emphysema, one at Aspen, Colorado, in June, 1958 (22) and the other in London in September, 1958 (3). Both recommend that fixation of the distended intact lung via the air passages should be performed before it is cut. The methods described here were illustrated in the London symposium (3). Each worker in this field will naturally select methods most suitable to his special enquiry. When large surveys are attempted, however, some standardization of fixation methods will be needed as well as rapid ways of identifying and grading emphysema. It is hoped that some of the suggestions in this article will be of interest to both the large-scale researcher and the solitary pathologist who wants to see emphysema for himself.

Summary

In previous communications on the pathology of pulmonary emphysema, it was suggested that

lungs should be fixed under a continuous pressure of 25 to 30 cm. of aqueous formalin for seventy-two hours to prevent shrinkage and distortion (pressure fixation). In the present communication, details are given of an improved apparatus for this purpose, together with further reasons for the procedure and further evidence to suggest that the volumes are comparable approximately to full inspiration in life. If followed by barium-sulfate impregnation of lung slices, pressure fixation enables the pathologist to detect emphysema of all varieties. Technical assistance is not essential.

Sumario

Histopatología del Enfisema Pulmonar: Métodos de Estudio

En comunicaciones anteriores relativas a la histopatología del enfisema pulmonar, se apuntó que debían fijarse los pulmones bajo continua presión de 25 a 30 cm. de formalina acuosa durante setenta y dos horas para impedir la contracción y deformación (fijación por presión). En la comunicación actual, se ofrecen pormenores de un aparato perfeccionado para dicho fin, nuevas razones en pro del procedimiento y nuevos datos indicativos de que los volúmenes son comparables aproximadamente a los de la plena aspiración en la vida. Si va seguida de la impregnación de cortes pulmonares en sulfato de bario, la fijación por presión capacita al patólogo para descubrir el enfisema de cualquiera variedad. La ayuda técnica no es indispensable.

Resume

Histopathologie de l'emphysème pulmonaire: méthodes d'étude

Dans des communications précédentes sur l'histopathologie de l'emphysème pulmonaire, on a suggéré que les poumons pouvaient être fixés par une pression continue de 25 à 30 cm d'eau formolée, pendant soixante douze heures, afin de prèvenir un rétrecissement et une distortion (fixation à la pression). Dans la présente communication, on donne des détails sur un procédé amélioré à cette intention, des raisons supplémentaires d'utiliser ce procédé, et une évidence supplémentaire qui fait penser que les volumes sont à peu près comparables à une inspiration complète du sujet en vie. Si la fixation à la pression est suivie par une imprégnation des coupes de poumon par du sulfate de baryum, elle permet à l'histopathologiste de détecter toutes les variétés d'emphysème. Une assistance technique n'est pas essentielle.

Acknowledgment

The writer is indebted to Professor C. V. Harrison, Dr. I. Doniach, and Dr. C. M. Fletcher for advice; to Dr. J. W. Laws for estimating the lung volumes roentgenographically; to Dr. G. Ball for recommending the centrifugal pump; and to Mr. W. Brackenbury for the photographs.

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LUNG NEEDLE BIOPSY IN THE EVALUATION OF DIFFUSE PULMONARY DISEASE[1, 2]

An Experimental Study

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Introduction

Needle biopsy techniques have been applied to the study of the histopathology of many organs and tissues, including liver, kidney, bone, and muscle. They have been found easy techniques and reliable diagnostic aids in various illnesses. While lung disease has been well described in terms of alteration of physiologic parameters, histologic studies have been less frequently used in the management of clinical problems. Needle biopsy of consolidated pneumonic areas, of intrathoracic and intrapulmonary masses adjacent to the chest wall, and of parietal pleura when pleural effusion is present, is considered a useful diagnostic procedure (1–4). In contrast, needle biopsy of diffusely involved lung parenchyma is generally considered an unsatisfactory procedure, frequently complicated by pneumothorax and bleeding (5).

The therapy currently available for clinical entities such as fungous infections, collagen diseases, tuberculosis, lymphomas, and nonspecific granulomas, necessitates an etiologic diagnosis. The precise demonstration of specific etiologic factors in the pneumoconioses has medico-legal import. At present, etiologic diagnosis of all of these diseases often requires thoracotomy. The method suggested by Klassen and associates, and now rather widely used, is still a major surgical procedure (6).

For these reasons a more accurate estimation of the risk involved in diagnostic percutaneous needle biopsy of lung tissue is desirable. This technique would seem to be especially applicable in diffuse nodular lesions and widespread vascular diseases of the lung. The contraindications to its use in cystic, blebous, or emphysematous diseases remain obvious. If the lung tissue can be made available without a major surgical procedure, and if serial specimens can be obtained through the course of an illness at no more than minimal risk to the patient, obvious advantages derive from this technique. Tissue so obtained would lend itself not only to study of microscopic pathology, but also could be used for histochemical, microchemical, bacteriologic, and immunologic observations.

The present study was undertaken to evaluate the risk of percutaneous needle biopsy of the lung in experimental animals. Technical problems inherent in the procedure were elucidated. The accuracy of differentiation between normal and experimentally altered lung tissue was evaluated.

Materials and Methods

A total of 58 lung biopsies was obtained from 22 dogs. Biopsies were taken, using a Vim-Silverman needle introduced into the animal's mid-lung field. The standard technique for biopsy of other tissues was used. Animals were anesthetized with sodium pentobarbital. Some were intubated with an endotracheal cannula and ventilated with a Harvard pump, so that the respiration could be arrested at any desired phase for the biopsy. Posteroanterior and lateral chest films were taken before and after each biopsy. In each animal, one or more subsequent biopsies with temporally related roentgenograms were taken at weekly intervals. The longest period of follow-up observation was three months, during which time a series of 12 biopsies and roentgenograms was made.

Biopsy specimens were placed immediately in 10 per cent formalin and subsequently examined microscopically after staining with hematoxylin and eosin. Some of the specimens were evaluated by viewing serial sections on film strips, using a special process recently developed at Duke University Medical Center (7). By this technique, the entire tissue removed can be studied, while portions of the film can be left unstained for subsequent special stains. Since adequate samples

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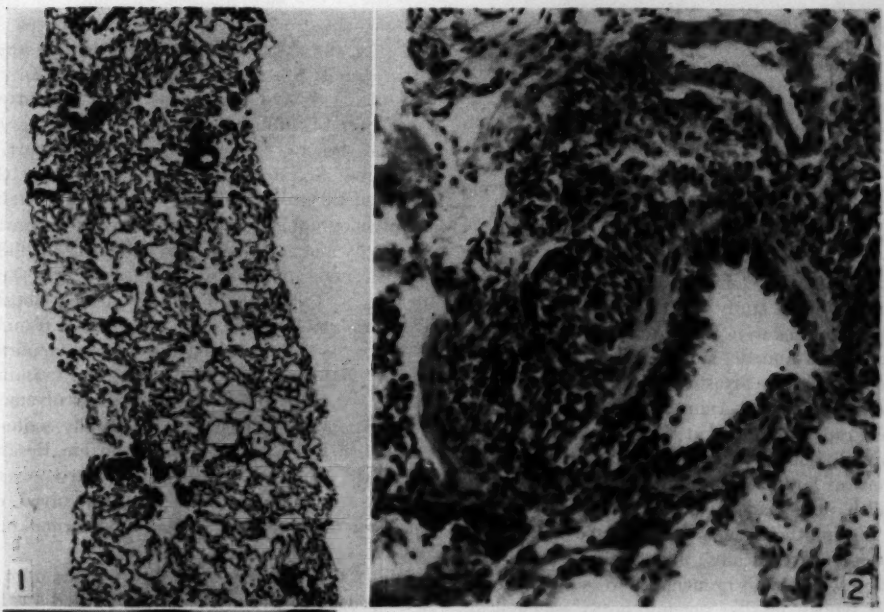

FIG. 1. Representative example of histologic findings in lung tissue obtained by percutaneous needle lung biopsy. Specimen measuring 8 mm. in length by 2 mm. in width. Normal lung. (× 84)

FIG. 2. Percutaneous lung biopsy specimen. Section of bronchiole; normal lung tissue. (× 228)

FIG. 3. Gross lung specimen showing several areas of fibrosis and scarring on the pleural surface from needle trauma.

were made available by the needle biopsy procedure, the standard slide method was found to be satisfactory for most of the histologic studies.

After baseline biopsy and roentgenograms, pulmonary lesions were induced by endobronchial administration of sulfur dioxide. Various amounts of 100 per cent sulfur dioxide gas were delivered manually in less than one minute into one lobe of one lung through a No. 6 Cournand catheter placed under fluoroscopic observation. The contralateral lung and, to a lesser extent, the remaining lobes of the ipsilateral lung, were protected by excess flow of oxygen threefold greater than the animal's minute ventilation delivered through a similar catheter placed in the opposite lung. A regular endotracheal tube with inflatable cuff was used to wedge the two catheters firmly in the trachea and to allow the collection of all expired sulfur dioxide gas into Douglas bags. Upon completion of the experimental treatment, the expired gas was ex-

hausted to the outside atmosphere. Weekly follow-up biopsies and roentgenograms were continued. Roentgenograms were made, utilizing the same technical factors for each animal. These films yielded comparative studies of the development and regression of gross changes and served as a guide in localizing the site for subsequent biopsies. Surviving animals were sacrificed after two or three months of serial studies, and necroscopy limited to heart and lungs was performed.

Observations

It was soon found that specimens suitable for histologic study were most easily obtained when biopsies were taken at the peak of inspiration. With animals mechanically ventilated, the respirator was arrested at this phase of the respiratory cycle. Animals breathing ad libitum were rapidly biopsied at this respiratory phase by carefully observing the chest excursion. Specimens as large as 8 mm. in length and 2 mm. in width were made available (figure 1).

Pneumothorax was not detected clinically or roentgenographically under these conditions. Even when small bronchioles were punctured by the biopsy needle, pneumothorax was not observed (figure 2). The only 2 instances of pneumothorax occurred in unsatisfactorily anesthetized animals with tachypnea or irregular breathing.

The chest was opened and the lungs were examined grossly in 15 animals at different periods of time after one or more biopsies were taken. No evidence of significant parenchymal or extrapulmonary hemorrhage was observed. Different stages of resolution of the small pulmonary parenchymal lesions produced by the introduction of the needle were frequently noted. These varied from minimal oozing of blood on the visceral pleura to pin-pointed areas of superficial fibrosis and scarring (figure 3).

Animals receiving in excess of three quarters of a liter of sulfur dioxide had diffuse parenchymal lesions, but died despite supportive measures. Six dogs survived exposure to the gas, and they all developed lesions demonstrable roentgenographically. These consisted primarily of patchy and scattered areas of density medially located and apparently not reaching the lung periphery. Two of the 6 animals yielded biopsies showing histologic evidence of chemical trauma. Biopsies in the remaining 4 dogs were reported as "normal lung tissue." Post-mortem examination revealed circumscribed areas of involvement extending to the pleural surface, chiefly confined within the lobe exposed to sulfur dioxide. Residua from the exploring needle were noted several centimeters from the areas grossly involved, explaining the histologic report of "normal lung tissue."

When the tissue specimen was obtained from an involved area, the alterations produced by the acute chemical injury were easily identified histologically. Table 1 is an abstracted protocol of one of the surviving animals, showing the temporal relationships of the sulfur dioxide exposure and the observed parameters of this study.

Discussion

Complications arising from percutaneous needle lung biopsy, pneumothorax in particular, were nonexistent in the writers' experience when

TABLE 1

Protocol Experiment No. 18

Date	Procedure	Findings
January 23, 1960	Roentgenogram (18 R 1)	Normal (figure 4)
January 23, 1960	Needle biopsy (18 S 2)	Normal (figure 5)
January 26, 1960	Right middle lobe exposed to 500 ml. of 100% sulfur dioxide	
January 28, 1960	Roentgenogram (18 R 3)	Dense consolidation in the right mid-lung field; prominent pulmonary artery (figure 6).
January 28, 1960	Needle biopsy (18 S 3)	Alveolar edema; vascular congestion; scattered neutrophilic infiltration of the alveolar walls scattered macrophages within the alveoli (figure 7).
March 23, 1960	Roentgenogram (18 R 7)	Normal (figure 8)
March 23, 1960	Needle biopsy (18 S 6)	Normal, except for scattered macrophages (figure 9)

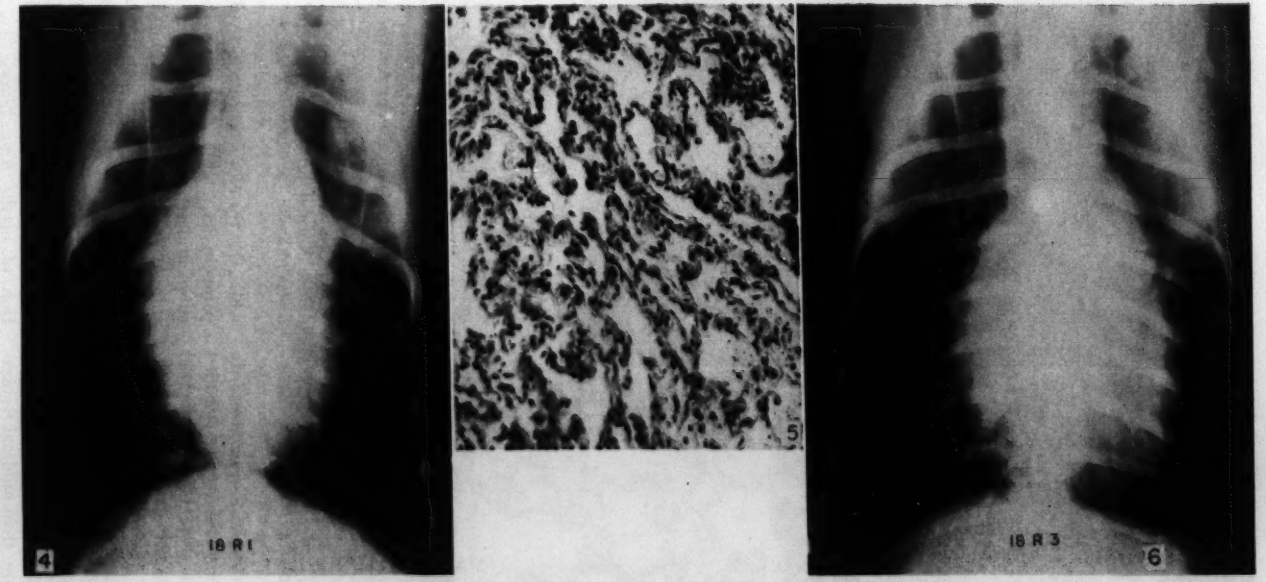

FIG. 4. Experiment No. 18. Baseline roentgenogram. Normal chest film.
FIG. 5. Experiment No. 18. Baseline needle biopsy. Normal lung tissue. (× 228).
FIG. 6. Experiment No. 18. Roentgenogram two days after sulfur dioxide. Dense consolidation in the right mid-lung field; prominent pulmonary artery.

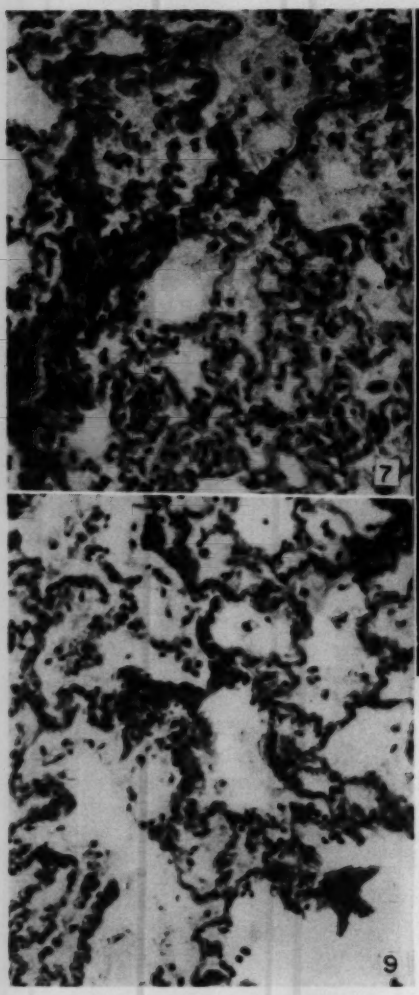

FIG. 7. Experiment No. 18. Lung needle biopsy two days after exposure to sulfur dioxide. Alveolar edema; vascular congestion. Scattered neutrophilic infiltration of the alveolar walls; scattered macrophages within the alveoli. (× 228).

FIG. 8. Experiment No. 18. Roentgenogram approximately two months after sulfur dioxide. Normal chest film except for prominence of the pulmonary artery.

FIG. 9. Experiment No. 18. Lung needle biopsy approximately two months after sulfur dioxide. Normal lung tissue except for scattered macrophages. (× 228)

biopsies were taken with respiration momentarily arrested. If the respiratory activity is suspended while the pleural surfaces are being perforated by the needle, intra-alveolar air may communicate with extrathoracic atmospheric air, but no significant leakage of gas is possible into the pleural cavity. The intrapleural adhesive force cannot be overcome by the inwardly directed force of the pulmonary elastic recoil. If during the biopsy procedure more than minimal respiratory motion is allowed, and particularly if irregular and forceful respiratory activity occurs while the tip of the needle is in the pulmonary parenchyma, large traumatic lacerations of subpleural septa and vessels may occur causing subsequent leakage of air and fluid into the pleural space. Tachypnea or irregular breathing which cannot be momentarily controlled should be considered a contraindication to percutaneous needle biopsy of the lung.

With respect to the second possible complication of needle lung biopsy, i.e., hemorrhage, under the present experimental conditions, neither intrapulmonary nor extrapulmonary

bleeding of any consequence was noted. If a normal coagulation mechanism exists, damage from the needle to the small blood vessels of the lung periphery is minimal and heals without untoward difficulty.

Fifty-one of the 58 specimens obtained by needle biopsy were found to be adequate for histologic study. Distortion of the normal alveolar architecture by compression from the needle's cutting edge was insignificant in almost all instances. While no chemical, bacteriologic, or sero-immunologic observations were made on the tissue obtained, the size of the specimens was considered suitable for such studies.

Histologic evidence of chemical trauma, or "positive" needle biopsy, was obtained in only 2 of the 6 animals surviving limited exposure to sulfur dioxide. Yet, all of the animals showed definite anatomic changes by roentgenographic and necroscopic examination. These lesions in the surviving animals were never diffuse enough to involve the whole lung field and tended to have a patchy distribution within the lobe primarily involved. Moreover, the histologic findings demonstrated at biopsy were considered "positive" only if they could be directly related to an acute chemical injury. Criteria for this consideration were intense congestion of alveolar capillaries, sludging and hemolysis of the erythrocytes, hemorrhage, edema, and alteration of cellular architecture of the alveolar walls. The present experimental conditions were, therefore, very unfavorable for ensuring "positive" biopsy reports in this group of diseased animals. However, for the purpose of this study, the lesions induced were adequate to show that, if the biopsy specimen is obtained from an involved area, the alveolar and vascular abnormalities can be demonstrated histologically. If widespread disease of the lung is present, its recognition by the use of this technique is likely.

Summary

The possible complications of percutaneous needle biopsy of the lung were evaluated. Fifty-eight biopsy specimens were obtained from 22 dogs with a regular Vim-Silverman needle. Pulmonary disease was experimentally induced with sulfur dioxide gas and was followed in 6 surviving animals with serial biopsies and roentgenograms at weekly intervals for periods up to three months. Animals were then sacrificed and control post-mortem examinations were performed.

Tissue specimens suitable for histologic studies were obtained without difficulty by taking the biopsy at the peak of inspiration, with respiration momentarily arrested. Complications such as pneumothorax or significant bleeding were not observed. The lesions produced by the sulfur dioxide in the surviving dogs were found to be less diffuse than anticipated. However, alveolar and vascular changes secondary to the chemical injury were recognized by percutaneous needle lung biopsy in 2 animals. On the basis of these observations, it is suggested that percutaneous needle lung biopsy is a reasonably safe and informative procedure. It should be considered as a diagnostic aid in patients with homogeneously diffuse pulmonary disease prior to diagnostic thoracotomy.

Sumario

La Biopsia Pulmonar con Aguja en la Justipreciación de las Enfermedades Pulmonares Difusas. Estudio Experimental

Se justiprecian las posibles complicaciones de la biopsia percutánea con aguja del pulmón. De 22 perros se obtuvieron 58 ejemplares biópsicos con una aguja corriente de Vim-Silverman. Se provocó experimentalmente enfermedad pulmonar con gas de bióxido de azufre, siguiendo los 6 animales sobrevivientes con biopsias seriadas y radiografías a plazos semanales durante períodos hasta de tres meses. Se sacrificó entonces a los animales, ejecutándose autopsias que sirvieron de testigos.

Ejecutando la biopsia en el acmé de la aspiración, con la respiración detenida de momento, se obtuvieron sin dificultad ejemplares de tejido apropiados para estudio histológico. No se observaron complicaciones tales como neumotórax o hemorragia de cuantía. Las lesiones producidas por el bióxido de azufre en los perros sobrevivientes resultaron ser menos difusas de lo esperado. Sin embargo, se reconocieron en 2 animales con la biopsia percutánea pulmonar con aguja alteraciones alveolares y vasculares secundarias a la lesión química. A base de estas observaciones, se sugiere que la biopsia percutánea del pulmón con aguja es un procedimiento bastante inocuo e informativo, que hay que considerar como auxiliar diagnóstico en los sujetos que padecen de enfermedad pulmonar difusa homogéneamente antes de verificar una toracotomía para diagnóstico.

Resume

Biopsie du poumon à l'aiguille dans l'évaluation de maladies pulmonaires diffuses. Une étude expérimentale

Les auteurs évaluent les complications possibles de la biopsie transcutanée, à l'aiguille, du poumon. Cinquante huit échantillons de biopsies furent obtenus chez 22 chiens, avec une aiguille ordinaire de Vim-Silverman. On occasionna expérimentalement une maladie pulmonaire avec du dioxyde sulfurique et il y eut par la suite, chez 6 animaux survivants, des biopsies en séries et des radiographies hebdomadaires pendant des périodes allant jusqu'à trois mois. Les animaux furent ensuite tués et on pratique des examens post-mortem.

Des échantillons de tissu convenables pour des études histologiques furent obtenus sans difficulté en prenant la biopsie au maximum de l'inspiration, avec la respiration momentanèment arrêtée. On n'observa pas de complication telle qu'un pneumothorax ou un saignement important. Les lésions produites par le dioxyde sulfurique, chez les chiens survivants, furent, trouva-t-on, moins diffuses qu'on ne s'y attendait. Cependant, chez 2 animaux, on reconnut grace à la biopsie transcutanée pulmonaire à l'aiguille, des changements alvéolaires et vasculaires secondaires à la lésion chimique.

En se basant sur ces observations, on donne à penser que la biopsie transcutanée pulmonaire, à l'aiguille, est un procédé raisonnablement sans danger, et instructif. Il devrait être considéré comme une aide pour le diagnostic chez des malades ayant une maladie pulmonaire diffuse d'une façon homogène, passant avant un diagnostic par thoracotomie.

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VASCULAR INVASION IN LUNG CANCER[1, 2]

Clinical-Pathologic Significance

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INTRODUCTION

Surveys of carcinoma of the lung have traditionally placed emphasis upon the involvement of the hilar lymph nodes (1, 2) and the cell type (3) as the major factors determining prognosis. It is the purpose of this presentation to show that the presence or absence of invasion of the pulmonary venous system by cancer cells is the most important single factor effecting survival in lung cancer. At the time the present work was begun, no series of surgically resected specimens had been studied correlating survival with invasion of the pulmonary veins. The writers theorized that the rich vascular bed of the lung would make the likelihood of vascular invasion by lung cancer of considerable importance.

Having been interested in this subject for many years, in 1954 a study was begun in the Santa Barbara Cottage Hospital of the surgically resected cases of lung cancer. After observing that some patients with minute lung cancers with negative hilar lymph nodes died within a relatively short period of brain or bone metastases, it became apparent that factors other than spread to the hilar nodes determined the prognosis.

Aylwin (4), in 1951, reported upon vascular invasion in surgically resected specimens of bronchogenic carcinoma. Collier and associates (5, 6), in 1957, studied blood vessel invasion in lung cancer in 226 patients who were subjected to pulmonary resection in a sixteen-year period ending in December, 1955. The incidence of blood vessel invasion for the entire group of cases was 71 per cent, while it was 100 per cent for undifferentiated carcinoma and 63 per cent for epidermoid carcinoma. In their series, most patients with blood vessel invasion died within two years and only 6 per cent survived five years, compared with a five-year survival of 75 per cent of those without blood vessel invasion. Ballantyne (7), in 1957, reported a series of cases of lung cancer resected between November, 1950, and December, 1951. Blood vessel invasion was found in 80 per cent of the squamous cell carcinomas, whereas the undifferentiated carcinomas and the adenocarcinomas had a 100 per cent incidence of blood vessel invasion. Langston (8), in 1958, studied 15 surgical specimens and found evidence of venous invasion in 14 of the 15 resected cases. In the one case that did not show evidence of blood vessel invasion, lymph node metastases were present.

MATERIAL AND METHODS

In the six-year period from January, 1954, through December, 1959, all primary lung neoplasms which had been treated by lobectomy or pneumonectomy were carefully studied to determine the presence of vascular invasion. In addition to the customary blocks of lymph nodes, the margins of surgical resection and pulmonary parenchyma, multiple blocks were obtained from the primary neoplasm. An average of 15 slides were studied per case with as many as 30 slides in several instances. In all of those in which vascular invasion was not clearly evident with the routine hematoxylin and eosin stains, the Verhoeff-Van Giesen stain was employed to demonstrate vascular elastica. The Alcian blue–periodic Schiff stain was occasionally used to establish histologic type.

The criteria of vascular invasion were standard. Free cells within vascular lumina and invasion of media with a still intact intima were not considered vascular invasion.

RESULTS

Venous invasion was found in 22 of 25 surgically resected cases of lung cancer, giving an incidence of 88 per cent; whereas arterial invasion was found in 11 of 25 cases, or 44 per cent. Lymph node metastasis was found in 12 of 25 cases (48 per cent). The incidence of the various types of tumors found in this series is given in table 1.

Only 3 of the 25 patients failed to show venous

[1] From the Santa Barbara Cottage Hospital, Santa Barbara, California.

[2] Presented before the Medical Session, as part of *Section 7A*, at the annual meeting of the American Trudeau (now Thoracic) Society, Los Angeles, California, May 18, 1960.

TABLE 1
TYPES OF TUMORS

Squamous cell carcinoma	15 of 25—60%
Adenocarcinoma	8 of 25—32%
Undifferentiated carcinoma	1 of 25— 4%
Cylindromatous carcinoma	1 of 25— 4%

TABLE 2
INCIDENCE OF SPREAD

Venous invasion	22 of 25—88%
Arterial invasion	11 of 25—44%
Lymph node metastasis	12 of 25—48%

TABLE 3
SURVIVAL AND VENOUS INVASION

Patients with Venous Invasion	Patients without Venous Invasion
12—dead 1 year, 54%	1—alive, 6 years 9 months
16—dead 2 years, 72%	1—alive, 2 years 8 months
19—dead 3 years 86%	1—died, alcoholism, 3 years 10 months

invasion in their specimens. One of these 3 patients is alive and well six years and nine months following a pneumonectomy, whereas one died of alcoholism three years and ten months after surgery with no evidence of recurrence of disease at the time of death. The remaining case is that of a patient with a cylindromatous carcinoma who is alive and well after two years and eight months. It is interesting that none of these patients, to date, have died of lung cancer.

The incidence of venous and arterial invasion and lymph node metastasis is depicted in table 2.

Of the 22 cases of venous invasion, 12 patients, (54 per cent) died within one year. In two years, 17 of the 22 had died, 16 (72 per cent) dying of carcinoma. Within three years, 19 of 22 (86 per cent) had died of carcinoma. Of the 2 survivors, one is alive and well two years and seven months later, whereas the other is alive and well two years and five months later without evidence of recurrence. The survival in this group with venous invasion is 9 per cent. Eight of the 19 patients who died of carcinoma had metastases to bone, brain, or central nervous system, an incidence of 42 per cent. The survival with and without venous invasion is shown in table 3.

Thirteen of 25 patients, or 52 per cent, had hilar nodes free from demonstrable cancer. Eight of the 13 patients without lymph node invasion are dead, 7 (53 per cent) dying of their disease. Six of these 7 patients died with metastasis to the central nervous system, bone, or brain. In the group that died of disease without lymph node metastasis, 7 (100 per cent) showed blood vessel invasion in the tissue specimens.

Twelve of 25 patients (48 per cent) had neoplastic changes in the hilar nodes. All 12 (100 per cent) had venous invasion and all died of carcinoma.

COMMENT

In the present series, if blood vessel invasion was present in the surgical specimen, 72 per cent of the patients died within two years and 86 per cent died within three years. Obviously, some cases of tumor lying within the pulmonary vein will not spread as tumor emboli via Batson's veins (9), vascular shunts, or the pulmonary vein to become implanted as tumor metastases. It is possible that the biologic predeterminism might govern whether such implants will actively grow as metastatic tumors. The resistance of the host is probably, and might well be, the determining factor as to whether or not invasion of the vascular spaces occurs. There is some correlation between the degree of malignancy and the incidence of blood vessel invasion. Vascular invasion may be an indication of the malignant potential, inasmuch as 100 per cent of the undifferentiated carcinomas in the present series have shown venous invasion. In the present series, neither the size of the lesion nor its location has had any influence upon the prognosis. It is believed that any manipulation of the lung during resection for lung cancer should be kept at a minimum and that a ligation of the pulmonary vein as an early step in the procedure may be important in preventing tumor embolization.

If the hilar lymph nodes contain neoplastic disease, it is doubtful if a more radical removal of the mediastinal nodes would effect the survival in lung cancer, as most all of the patients die of widespread metastases due to vascular invasion.

SUMMARY

Invasion of the pulmonary vein by tumor cells is the important factor determining prog-

nosis in lung cancer. Venous invasion occurs in a high percentage of cases of lung cancer. The presence of normal hilar lymph nodes does not imply a favorable prognosis. The survival rate is higher if venous invasion is absent.

Sumario

La Invasión Vascular en el Cáncer Pulmonar: Importancia Clínico-Patológica

La invasión de las venas pulmonares por células tumorosas es el importante factor que determina el pronóstico en el cáncer pulmonar. En un elevado porcentaje de los casos existe inversión venosa. La presencia de ganglios linfáticos hiliares negativos no denota un pronóstico favorable. El índice de sobrevivencia es más alto si no hay invasión venosa.

Resume

Invasion vasculaire dans les cancers du poumon: signification clinicopathologique

L'invasion de la veine pulmonaire par des cellules cancéreuses est le facteur important qui détermine le pronostic dans le cancer du poumon. L'invasion veineuse se produit dans un pourcentage élevé des cas de cancer du poumon. La présence de ganglions lymphatiques hilaires négatifs n'implique pas un pronostic favorable. Le taux de survie est plus élevé s'il n'y a pas d'invasion veineuse.

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THE EVOLUTION OF BULLOUS CAVITIES IN ADEQUATELY TREATED EXPERIMENTAL PULMONARY TUBERCULOSIS[1, 2]

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INTRODUCTION

Tuberculous cavities in which the inner wall consists of smooth fibrous connective tissue have frequently been observed since the advent of antituberculous chemotherapy. Altmann and Diaz (1) found 14 such "clean-walled" cavities among 157 surgically resected lungs and at 91 autopsies. The patients had had cavitary tuberculosis treated with isoniazid. Altmann and Diaz suggested that the "healing" of the tuberculous cavities might be a distinctive effect of isoniazid since they had found only one similar structure among 193 patients with cavitary tuberculosis treated with streptomycin. However, they did not indicate the intensity or the duration of streptomycin therapy.

Although the occurrence of thin-walled tuberculous cavities is not rare, it is unusual to observe them regress and disappear on serial roentgenographic examinations. In 1955, only 25 such thin-walled, relatively rapidly evolving and regressing air spaces had been culled from the literature by Alarcon (2), particularly from the European and South American literature. On the large pulmonary disease service at this hospital, only two such cases have been seen during the past seven years. When such large, thin-walled, air spaces have been seen to regress, there has been controversy as to whether they represented peripheral obstructive emphysema due to distension and rupture of air spaces, that is, bullae; or whether they represented excavated cavities which might have become inflated due to some undemonstrated ball-valve mechanism. An opportunity to make a contribution to the understanding of these air spaces, for which the noncommittal term pneumatocysts is used in this report, presented itself during a study of experimental tuberculosis in rabbits.

Pneumatocysts have frequently been observed in rabbit tuberculosis treated with chemotherapy (3, 4). Bell (4) believed that the pneumatocysts observed roentgenographically in his 12 rabbits were caused by peripheral obstructive emphysema due to inflammation of the bronchioles or material within their lumina, but he did not demonstrate such bronchiolar changes in his photomicrographs. He was uncertain about the ultimate fate of the lesions, since no animal had been followed for more than four months. Caffey (5), on the other hand, believed that, when necrotic pulmonary foci (whether due to staphylococcic pneumonia, tuberculosis, or other cause) were liquefied and evacuated, the resultant cavity become increasingly expanded with air. This view was shared by many, including Bernou and Brun (6, 7) and Sandler (8), who thought that the subsequent enlargement of the cavity might be due to an endobronchial lesion or to extrinsic compression or distortion; but no proof was offered.

Steenken and Wolinsky (3) postulated a check-valve mechanism due to bronchial obstruction by inflammation and edema. They observed that the pneumatocysts disappeared roentgenographically, and examination of the lungs later suggested that the cysts had been replaced by linear scars. They attributed this to the subsidence of inflammation and the reestablishment of bronchial communication. They stated that the formation of cysts in experimental infection occurred after treatment with a number of drugs and was not a distinctive effect of isoniazid. Alarcon (2), reporting a case of spontaneous pneumothorax, took exception to the check-valve mechanism. He believed that the bronchocavitary junctions became epithelized, the bronchi remaining widely patent, and that pleural adhesions to the chest wall in some way caused enlargement of the cavities.

The following report comprises observations

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[2] This research was supported in part by Research Grant E-1758 from the Institute of Allergy and Infectious Diseases of the National Institutes of Health, U. S. Public Health Service.

on the development and evolution of giant pneumatocysts during intensive chemotherapy of pulmonary tuberculosis in rabbits infected with aerosolized bovine tubercle bacilli. It is divided into two parts. Part I was an experiment designed to study the culturability of tubercle bacilli after prolonged chemotherapy and the observations on pneumatocysts were incidental. Part II was specifically designed to disclose the pathology of pneumatocysts during the first four weeks of chemotherapy.

Part I

Materials and Methods

A group of 72 all white, male, New Zealand rabbits with an average weight of 3 kg. were infected in a Wells "infecting chamber" with aerosolized bovine tubercle bacilli, Ravenel VA strain, according to the technique described by Wells (9). The rationale of this method was to obtain uniform infection, but, unfortunately, technical difficulties prevented this. Therefore, six weeks after infection every animal was examined roentgenographically to detect the extent of infection. Lesions were classified as minimal, moderately advanced, and far advanced. Two animals in each group thus classified roentgenographically were sacrificed to determine the extent, number, size, and distribution of the pulmonary lesions. One rabbit died of nontuberculous pneumonia two weeks after infection.

The remaining 65 rabbits were treated with 100 mg. of streptomycin[3] and 24 mg. of isoniazid[3] in a single daily intramuscular injection. The combined drug therapy was continued without interruption for a year. During this time, 14 animals died. The 51 surviving rabbits were then divided at random into two groups. One group of 25 rabbits was sacrificed, and the size, number, and morphology of the residual lesions in each animal were determined. The remaining 26 rabbits received no further chemotherapy and served as controls. The purpose of this experiment was to attempt to relate the survival of the controls to the culturability of bacilli in the rabbits that had been sacrificed.

During the twenty-four months after the cessation of chemotherapy, 4 of the controls died of tuberculosis and 9 rabbits died of nontuberculous causes. All animals which died or were sacrificed were autopsied; the lungs were inflated by gravitational flow of formalin, and were subsequently dissected, blocked, sectioned, and stained by hematoxylin and eosin, Masson, and Ziehl-Neelsen stains. In 2 cases, whole lung preparations were processed on paper, prepared by means of a modified Gough and Wentworth technique. Representative lesions were selected for bacteriologic studies, the results of which will be published separately.

[3] Kindly supplied in combination as Streptohydrazid by Charles Pfizer & Co., Inc.

TABLE 1

Correlation between the Roentgenographic Classification and the Number of Pulmonary Lesions

Animal	Roentgenographic Classification prior to Treatment	Total Number of Lesions
B8	Minimal (Group A)	55
B (7 + 8)	Minimal (Group A)	36
C4	Moderately advanced (Group A)	71
C7	Moderately advanced (Group A)	49
B4	Far advanced (Group B)	179
C (9 + 10) A	Far advanced (Group B)	83

Results

In the 6 untreated animals which were sacrificed six weeks after infection, the number, size, and distribution of the lesions corresponded well to the interpretation of roentgenograms. The 2 rabbits with roentgenographically demonstrable minimal disease averaged 45 lesions; the rabbits with moderately advanced disease, 60 lesions; and the rabbits with far advanced disease, 131 lesions (table 1). Since there was relatively little difference between the animals with minimal and moderately advanced disease, they are combined and referred to as *Group A*. *Group A* comprised about 50 per cent of the animals. The remaining 50 per cent had far advanced disease shown roentgenographically and are referred to as *Group B*. It was thus established with high probability that all exposed animals had been infected. Roentgenograms of the animals suggested that about half had 50 (plus-minus 25) pulmonary lesions at the start of chemotherapy and that the remainder had a greater number of lesions.

The untreated animals sacrificed six weeks after infection showed no evidence of cavity formation (figure 1). The necrotic tuberculous lesions had large surrounding inflammatory zones, and free communication between the lesions and small bronchi was easily demonstrated (figure 2).

The interest of the present writers in the pathology of pneumatocysts in experimental tuberculosis resulted from inspection of the lungs of 5 rabbits that died of tuberculosis during the early months of chemotherapy (twenty-

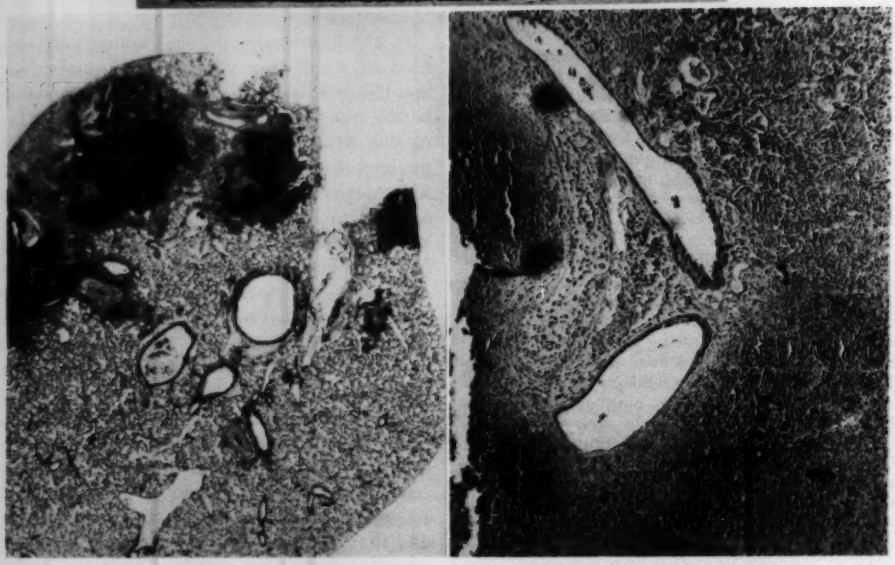

FIG. 1 (Upper). Control rabbit B4, six weeks after infection. Roentgenographic classification, *Group B*. Gross photograph showing many lesions measuring up to 1 cm. in diameter. No cavities are present.

FIG. 2*A* (Lower left). Photomicrograph of lung of rabbit B4, showing necrotic foci surrounded by inflammatory zones. (× 6) *B* (Lower right). Higher power of 2*A*, showing ulcerated bronchioles adjacent to necrotic foci. (× 48)

ninth to sixty-sixth days) (table 2). The first three animals: B (7 + 8) A; B (1 + 2) A; and B (9 + 10), died at the end of the first month. Their lungs contained numerous massive pneumatocysts (figure 3), the cavitary nature of which was suggested by yellow, necrotic masses adherent to the walls and projecting into the lumina (figure 4). The cysts remained in an inflated state despite collapse of the rest of the lung. A gravity flow of formalin entered them readily. The fourth rabbit (C6A) also had large cysts, one of which had ruptured. The fifth

TABLE 2

INCIDENCE OF INFLATED CAVITIES IN ANIMALS THAT DIED DURING CHEMOTHERAPY

Animal	Days of Therapy before Death	Roentgenographic Classification Prior to Treatment	Total Number of Lesions	Total Number of Inflated Cavities
		Group		
B (7 + 8) A	29	B	109	59
B (1 + 2) A*	30	B	202	34
B (9 + 10)	31	B	97	25
C 6A*	55	B	74	23
C (1 + 2) A*	66	B	150 estimated	100 estimated
C (7 + 8) A	114	A	35	2
C 8A	138	A	26	0 (1 collapsed)
B 4A	172	B	31	3 (4 collapsed)
C 6	265	B	39	1 (2 collapsed)
B 2	270	A	48	3
C 3A*	315	B	39	7
C 2A	318	B	80	0 (1 collapsed)
C (5 + 6) A	332	A	18	0
B 11	344	A	14	1

* Ruptured cavities detected in the formalin-inflated specimen.

rabbit (C (1 + 2) A) represented the maximal extent of pneumatocystic formation. There were approximately 100 inflated lesions in which the entrapped air readily mixed with formalin under gravity flow. The lung was literally converted into a mass of thin-walled, giant pneumatocysts (figure 5). These were lined by tuberculous granulation tissue, establishing their cavitary nature. Broken septa containing bronchi projected into the cavities (figures 6 and 7). Elongated tortuous bronchi (figure 8) surrounded the walls of the cavities, closely resembling the bronchi in emphysematous lungs demonstrated in plastic injection and corrosion technique by Liebow (10). Forty-six sections of 8 blocks of tissue were prepared. In these, bronchi could be demonstrated to enter the cavities in a devious, oblique fashion (figure 9). In untreated animals, in contrast, bronchi invariably communicated directly with the necrotic lesions. The manner in which the bronchi entered the cavities of the treated animals suggested that such anatomic structures could function as flap-valves.

In decided contrast to the findings in these 5 rabbits were 9 animals that died between the 114th and 344th days of chemotherapy. These showed few inflated cavities (figure 10). The rabbit (C8A) which died in the fifth month of treatment had a collapsed, subpleural lesion, which histologically contained a focus of calcified, necrotic material in which acid-fast bacilli were present (figure 11). Thereafter, the pleural surfaces of the lungs displayed a number of slightly sunken lesions, consisting of a fine, stellate network of gray strands, some of which extended more than 3 cm. in length (figure 12). On section, these still contained remnants of partially calcified, necrotic material.

Twenty-five rabbits were sacrificed at the end of a year of chemotherapy. Only 6 showed inflated cavities. In 4 of the 6 there was but a single pneumatocyst; in one rabbit there were 3 and, in another, there were 5. Numerous sunken, stellate, lesions were in evidence (figure 13).

Very few pneumatocysts were observed in the group of 26 rabbits that served as controls after the conclusion of one year of chemotherapy (table 3). This group was observed for twenty-four months. During that time half the group died, 4 of progressive tuberculosis and 9 of nontuberculous causes. The last animal to die of progressive tuberculosis succumbed ten months after the cessation of therapy. One animal autopsied a year later showed a small, collapsed cavity and a larger, encapsulated, necrotic focus containing acid-fast bacilli, closely resembling the encapsulated, necrotic foci found in human beings on resection after chemotherapy (figure 14). Twenty-

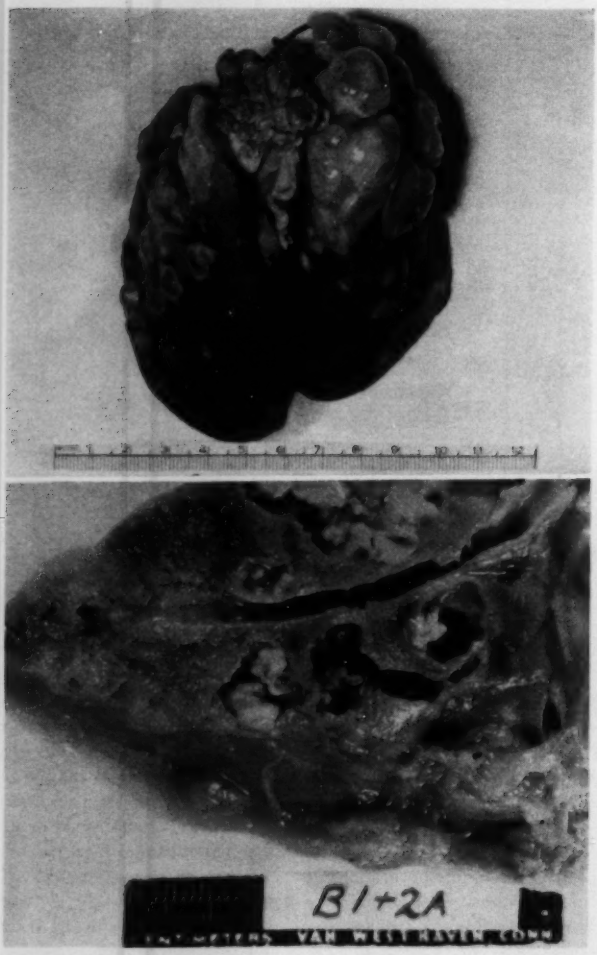

FIG. 3 (Upper). Rabbit B (7 + 8) A, dead on the twenty-ninth day of chemotherapy. Roentgenographic classification, *Group B*. Gross photograph, showing massive pneumatocysts replacing most of the right upper lobe with multiloculated cavities measuring up to 4 cm. in diameter.

FIG. 4 (Lower). Rabbit B (1 + 2) A, dead on the thirtieth day of therapy. Roentgenographic classification, *Group B*. Gross photograph, showing necrotic masses adherent to the cavity walls. The lungs were partly collapsed, with extensive adhesions at the apices. In the right lower lobe, an 0.8-cm. lesion had ruptured into the pleural cavity and a 3-mm. mass of caseous material extended into the space. From this mass a luxuriant growth of tubercle bacilli was obtained.

six months after therapy another rabbit died of nontuberculous causes, and a partly inflated lesion was present, associated with a small, calcified, necrotic focus.

PART II

Observations just cited revealed that inflated cavities were absent in untreated animals sacrificed six weeks after infection, while they were

numerous in the animals which died after one month of effective chemotherapy. There were no observations before the twenty-ninth day of chemotherapy, and the material did not clearly reveal the mechanism by which the cavities evolved. An experiment was therefore then designed to study the pathogenesis of inflated cavities during the first four weeks of chemotherapy.

Materials and Methods

A group of 24, all white, male, New Zealand rabbits, approximately 6 pounds in weight, were individually exposed to an infective aerosol in a Blickman bacteriologic hood. The aerosol was administered by the technique described by Steenken and Wolinsky (3). A fourteen-day-old culture, Ravenel (Trudeau), grown in Dubos Tween® albumin medium, diluted 1:100 (average 4–5 AFB/HPF) was aerosolized by means of a DeVilbiss hand atomizer into a polyethylene cylinder which served as a mask for the animal's face. Each animal received 5 puffs of the atomizer at approximately three- to five-second intervals, and the mask remained in place for an additional thirty seconds. Seven weeks after infection by the aerosol technique, 2 rabbits were selected at random and sacrificed to determine the number, size, and distribution of the lesions. Two rabbits did not survive the infecting period. Twelve rabbits were then started on drug therapy with daily streptomycin and isoniazid intramuscularly. The remaining 8 infected rabbits were not treated, and served as controls. At weekly intervals, 3 each of the treated rabbits and 2 each of the control rabbits were sacrificed, and the extent of pulmonary disease was investigated.

Results

Among the controls, the average number of lesions per rabbit was 27, with a range of 18 to 43; among the treated rabbits, the average was 31, with a range of 10 to 67 (table 4). It was apparent that there was excellent uniformity and comparability among the lesions established by this simple infective technique. Inflated bullous lesions were absent from the controls observed for as long as eleven weeks post infection. In the necrotic lesions of these animals, bronchial junctions were easily demonstrable.

The 3 rabbits sacrificed after one week of therapy had 10 to 67 lesions, only one of which was partially cavitated. Bronchial junctions with the necrotic foci were easily found.

The 3 rabbits sacrificed at the end of two weeks of therapy had 20 to 45 lesions. Two of them had

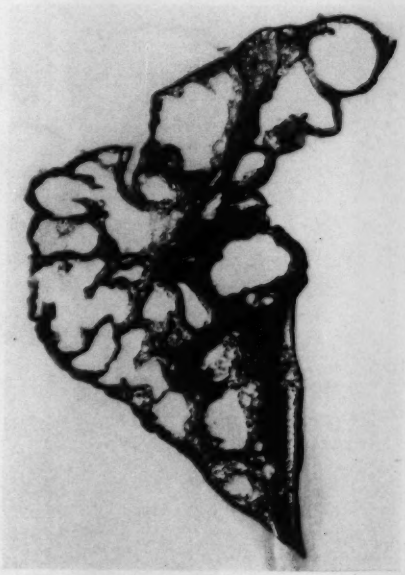

FIG. 5. Rabbit C (1 + 2) A, dead on the sixty-sixth day of therapy. Gross photograph of whole lung preparation on paper, showing necrotic masses, appearing here as white tissue, projecting into the cysts. Roentgenographic classification, *Group B*. The lungs were almost completely inflated, including about 100 pneumatocysts.

inflated cavities: one rabbit had one inflated cavity and the other had 10 cavities. Bronchocavitary junctions were still easy to find.

The 3 rabbits sacrificed after three weeks of therapy had 17 to 66 lesions and 2 to 27 inflated cavities (figure 15). Microscopically, these were lined by calcified, necrotic material and macrophages. The center of one large, necrotic nodule was replaced by protein-rich fluid. It was clear that the pneumatocysts which projected above the surface of the lung were not emphysematous blebs, but were, in fact, cavitated necrotic foci that had become inflated. Sixteen sections prepared from one such inflated cavity demonstrated the presence of an obliquely entering bronchiole, creating a flap-valve (figure 16). This appeared to be the anatomic basis for inflation of the cavities.

The 3 rabbits sacrificed after four weeks of therapy had 20 to 33 lesions, 5 to 6 of which

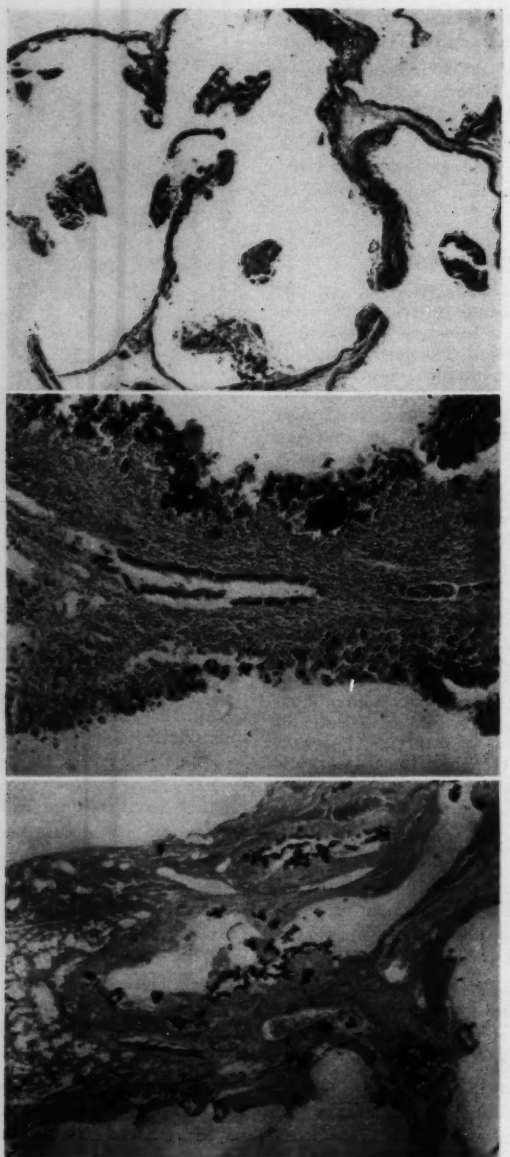

FIG. 6 (Upper). Rabbit C (1 + 2) A. Pneumatocysts with broken septa, lined by tuberculous granulation tissue. (Photomicrograph, × 6)

FIG. 7 (Center). Higher power photomicrograph of figure 6. Bronchi are present in septum between pneumatocysts lined by calcified granulation tissue. (Photomicrograph × 48)

FIG. 8 (Lower). Rabbit C (1 + 2) A, showing elongated compressed bronchus. (Photomicrograph × 6)

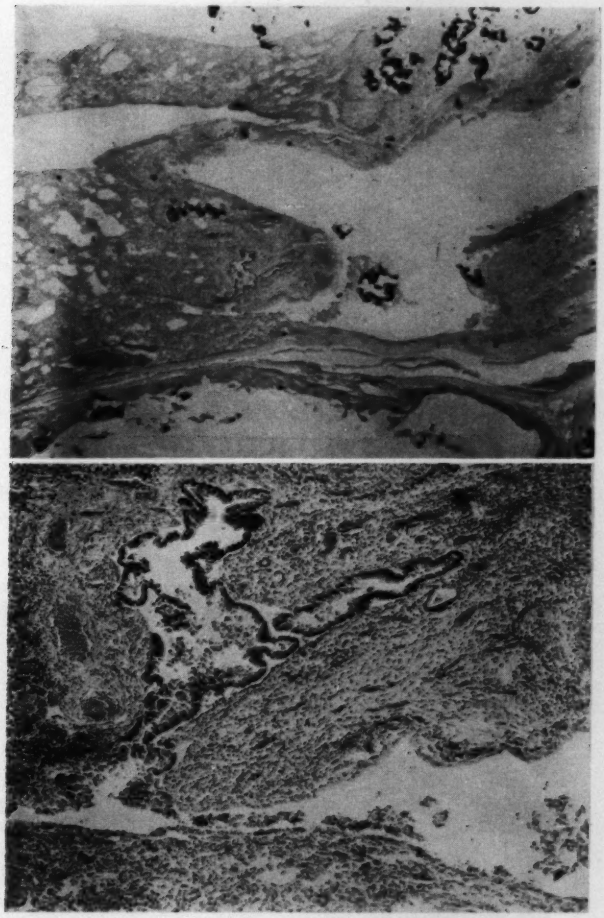

FIG. 9*A* (Upper). Rabbit C (1 + 2) A. Photomicrograph of pneumatocysts. A small amount of free, calcified, necrotic material is present at the bottom of the central cavity. To its left is a channel through which a bronchus enters the cavity indirectly, creating a flap-valve. (× 6) *B* (Lower). Higher-power photomicrograph of indirectly entering bronchus and flap-valve. (× 33)

were inflated cavities (figure 17). It was still possible to find bronchocavitary junctions in desultory sections of noninflated cavities.

DISCUSSION

In this study, 96 rabbits were exposed to infective aerosols of bovine tubercle bacilli. The initial experiment in which 72 rabbits were used was designed to yield bacteriologic data. The anatomic findings were a by-product. The rabbits were infected in a Wells' "infecting chamber," which did not result in a uniform number of pulmonary lesions. They were therefore examined roentgenographically and classified on the basis of the roentgenographic findings six weeks after infection. At this time, 6 animals were sacrificed and found to have extensive pulmonary tuberculosis compatible with the roentgen-

FIG. 10. (Rabbit C (7 + 8) A, dead on the 114th day of therapy. Roentgenographic classification, *Group A*. Gross photograph showing the two cavities in the left lower lobe, one of which contained a small, yellow, necrotic mass.

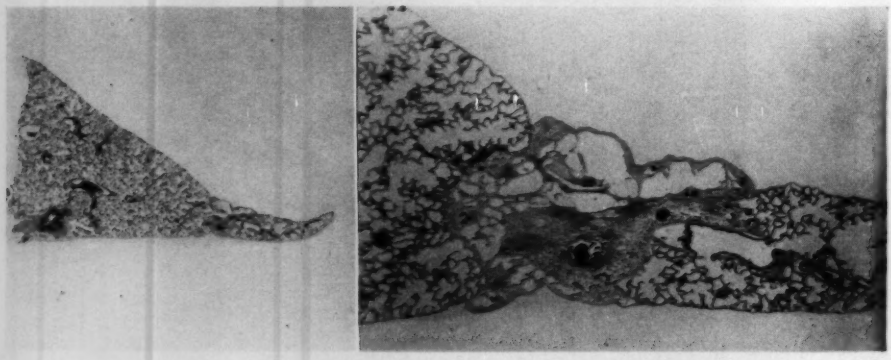

FIG. 11*A* (Left). Rabbit C8A, dead on the 138th day of therapy. Roentgenographic classification, *Group A*. Isolated, 2–4-mm. yellow, encapsulated foci were scattered throughout the lungs. Photomicrograph showing such a focus to the left and a collapsed, bleblike lesion to the right. (× 6) *B*. (Right). Higher power photomicrograph of 11*A* showing the partly collapsed pneumatocyst. (× 30)

ographic findings. One animal had died earlier of pneumonia. The remaining 65 animals were subjected to intensive chemotherapy for a year. During the year of therapy, 14 animals died, the earliest on the twenty-ninth day of treatment. Eleven of the 14 had inflated pneumatocysts. The 5 animals which died during the first two months of chemotherapy had, with one exception, the largest number of lesions. All showed a mass of inflated pneumatocysts, reaching a maximum of 100 in the animal that died on the sixty-sixth day of therapy. The lesions which grossly resembled huge bullae were demonstrated to be excavated tuberculous cavities, to the walls of which a zone of granulation tissue, necrotic debris, calcium salts, and tubercle bacilli clung tenaciously.

The results oppose the conclusions of Bell (4),

FIG. 12. Rabbit C3A, dead on the 315th day of therapy. Roentgenographic classification, *Group B*. Gross photograph showing thin-walled inflated cavities to the right and collapsed irregular pneumatocysts to the left.

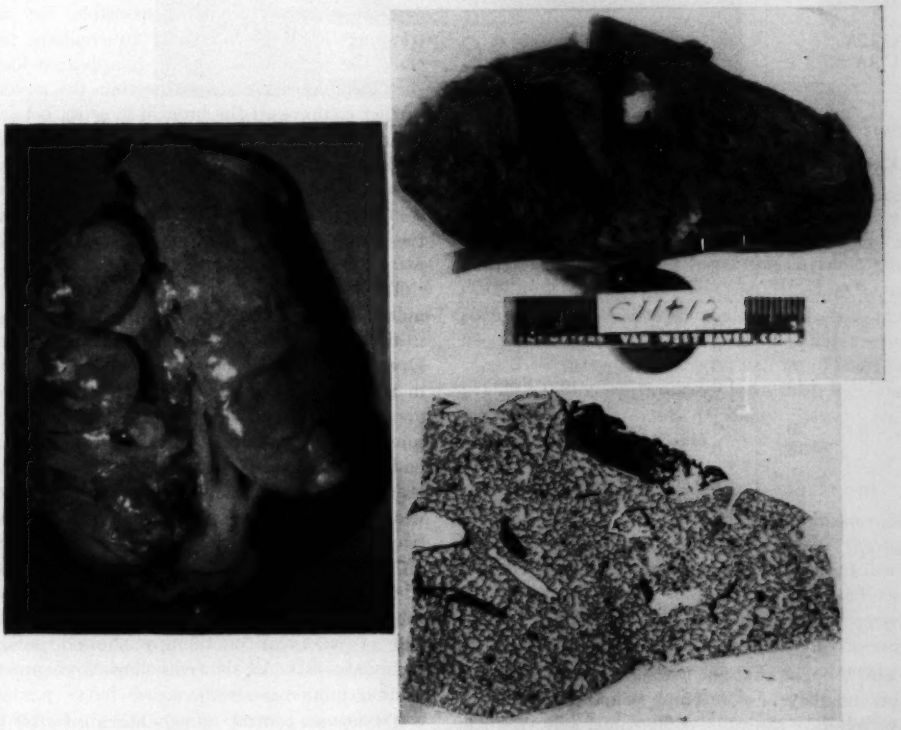

FIG. 13 (Left). Rabbit B (1 + 2), sacrificed after one year of chemotherapy. Gross photograph of both lungs showing thin-walled, bleblike pneumatocysts on the left, and stellate sunken lesions to the right.

FIG. 14*A* (Upper right). Rabbit C (11 + 12), dead two and one-half years after the start of chemotherapy. Gross photograph showing an encapsulated, yellow, necrotic focus above and the thin wall of a collapsed pneumatocyst below. *B* (Lower right). Photomicrograph of the encapsulated nodule in 14*A*. (× 6)

TABLE 3

PERSISTENCE OF INFLATED CAVITIES AFTER ONE YEAR OF CHEMOTHERAPY

Animal	Months of Survival after Termination of Chemotherapy	Cause of Death		Inflated Cavities
		Progressive Tuberculosis	Nontuberculous Disease	
C 10	<2		×	1
C 1	<2		×	0
B 6A	7+		×	0
B (9 + 10) A	7+	×		1
C 3	8+	×		0
B (11 + 12) A	9+		×	0
C 10A	9+	×		0
C 5A	10	×		0
C (5 + 6)	13+		×	0
C (11 + 12)	15+		×	2 collapsed
B 1	16+		×	1 collapsing
C 12A	18		×	0
C 4A	21+		×	0

TABLE 4

EARLY DEVELOPMENT OF INFLATED CAVITIES IN TREATED RABBITS

Weeks post Infection	Untreated Controls		Weeks of Treatment	Treated Rabbits	
	Primary Lesions	Cavitary Lesions		Primary Lesions	Cavitary Lesions
7	34	0	—	—	—
	24	0	—	—	—
8	18	0	1	67	0
	42	0		19	1
	—	—		10	0
9	25	0	2	20	1
	18	0		45	10
	—	—		26	0
10	24	0	3	66	27
	20	0		17	7
	—	—		24	2
11	23	0	4	33	5
	43	0		20	6
	—	—		22	6

who thought that such experimentally produced pneumatocysts were emphysematous blebs, but corroborate the work of Steenken and Wolinsky (3). Generally, necrotic calcified masses projected nipple-like into the cavities from one or more foci. Masson stains demonstrated little surrounding fibrosis. The thin, broken septa which projected into the multiloculated cavities in the most severe instances, contained bronchi or bronchioles which were free of necrotic material. Bronchi and bronchioles were easily found on random sections entering the necrotic foci of the untreated control rabbits or in those in the early stages of treatment.

Although the cavities remained inflated when the rest of the lung collapsed, suggesting the presence of air-trapping, a gravity flow of formalin through the trachea visibly entered the cavities and mixed with the air contents, indicating that bronchial communication did indeed exist. Several sections of blocks of tissue showed distorted bronchi and revealed, moreover, that some bronchi entered the cavities in a devious or oblique fashion. It therefore seemed likely that distortion of these bronchi, creating anatomic flap-valves, was chiefly responsible for air-trapping in the excavated, tuberculous foci. Once the process had begun, it appeared likely that the increasing tension within the cavities further compressed the bronchi in septa between them, accelerating the process to produce an explosive appearance of giant pneumatocysts. It seemed unlikely that necrotic material within the bronchi or bronchioles was responsible, since these were not found to be obstructed by such material.

The 9 animals which died during the last ten months of treatment showed a marked reduction in the number of macroscopic lesion of all kinds. Even more striking, however, was the reduction in the number of inflated cavities. These specimens showed evidence of the regression of previous pneumatocysts. They had collapsed and had become converted into stellate, nonaerated lesions consisting of small foci or calcified, necrotic material, still containing acid-fast bacilli. The collapse of inflated cavities occurred after the second month of therapy (*see* table 2). This is further substantiated by the observation that only 6 of the 25 animals sacrificed at the end of one year of therapy showed inflated pneumatocysts. All showed collapsed pneumatocysts or numerous stellate scars.

Twenty-six control animals remained after the termination of chemotherapy. Four of these died of progressive tuberculosis within ten months. Nine died of nontuberculous causes within twenty-four months after therapy was stopped.

FIG. 15. Gross photograph of rabbit lungs from animal sacrificed three weeks after the start of therapy, showing pneumatocysts. Left: cut surface; right: pleural surface.

Very few inflated pneumatocysts were found in this group of 13 rabbits. One of these animals autopsied at twenty-two months showed only small, fine scars and an encapsulated, necrotic focus. Another animal, dead of nontuberculous cause and autopsied at twenty-six months, showed a residual, partly collapsed pneumatocyst.

A second experiment designed to study the evolution of inflated cavities during the first month of chemotherapy confirmed and extended the observations in Part I. No inflated cavitary lesions occurred in the untreated control animals. In these, bronchial junctions with necrotic foci were easily found. In the treated animals, on the other hand, necrotic foci cavitated and became inflated between the second and third weeks of chemotherapy (*see* table 3). Bronchocavitary junctions were difficult to find. At the end of three weeks of therapy, serial sections were made of an inflated cavity, and an obliquely entering bronchus was demonstrated, creating a flap-valve as in the first group of animals. Such flap-valves, dependent upon distortion of bronchi and rapid and almost complete evacuation of necrotic material from a pneumonic focus, may well form an anatomic prerequisite for inflation. This may be a basic mechanism for the formation of pneumatocysts seen in acute clinical conditions in man, such as staphylococcic pneumonia (5). In this connection, it should be pointed out that flap-valves caused by other mechanisms, i.e., rapid changes in intrapulmonary pressure, have been found in association with—and probably cause—centrolobular emphysema (11).

SUMMARY

It has been shown that giant pneumatocysts which appear explosively, then disappear, during intensive chemotherapy of pulmonary tuberculosis in the rabbit are, in fact, inflated excavated necrotic foci. Excavation begins early, and inflated cavities are already present between the second and third weeks of chemotherapy. Anatomic flap-valves in the walls of the inflated cavities may be responsible for air-trapping, possibly accentuated by pressure on bronchi between cavities. This mechanism would appear to be a basic one and may account for pneumatocysts occurring in man when necrotic pulmonary

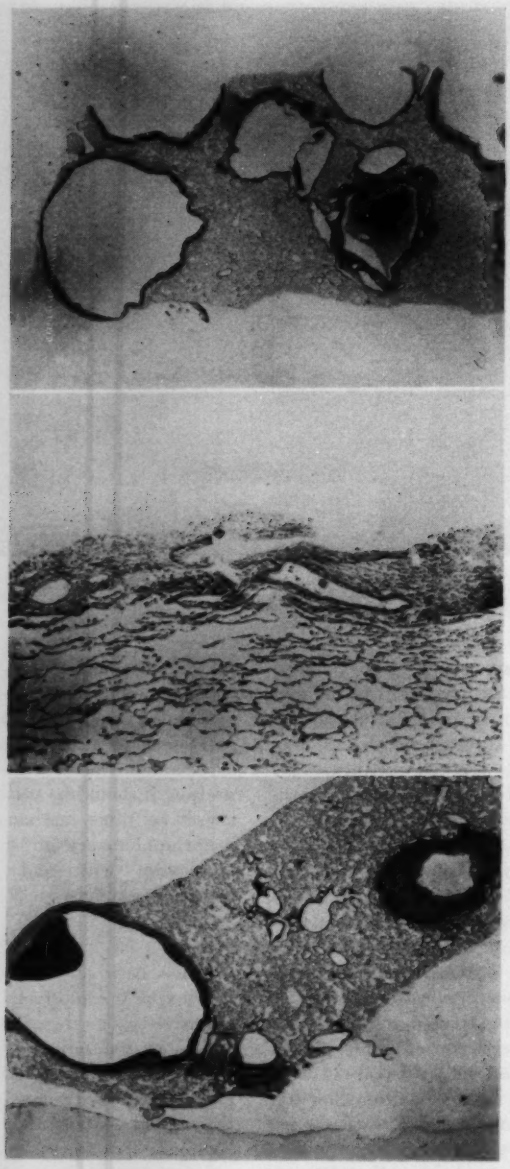

FIG. 16A (Upper). Photomicrograph of figure 15 showing discrete pneumatocysts. (× 6) *B* (Center). Higher power photomicrograph of 16A, showing obliquely entering bronchiole and flap-valve. (× 120)

FIG. 17 (Lower). Photomicrograph of rabbit lung from animal sacrificed four weeks after the start of therapy. A well-developed pneumatocyst projects above the pleural surface. Beneath the focally thickened pleura, a residual nubbin of calcified, necrotic material projects into the cavity. (× 10)

foci are rapidly and almost completely evacuated, as in staphylococcic pneumonia. In rabbits, the inflated cavities reached their maximal number at the end of the second month of therapy; after this, there was irregular deflation and collapse into small irregular structures surrounding residua of necrotic material, although a partly collapsed pneumatocyst was still found to persist two years after the termination of chemotherapy.

Sumario

La Evolución de las Cavernas Vesiculares en la Tuberculosis Pulmonar Experimental Tratada Adecuadamente

Se ha demostrado que los neumatoquistes gigantes que aparecen explosivamente y luego desaparecen, durante la quimioterapia intensa de la tuberculosis pulmonar en el conejo, son, de hecho, focos esfacelados, excavados, inflados. La excavación comienza pronto y ya hay cavernas infladas entre la segunda y la tercera semanas de la quimioterapia. Chapaletas anatómicas localizadas en las paredes de las cavernas infladas pueden ser la causa del atrape de aire, posiblemente acentuado por la presión ejercida sobre los bronquios entre las cavernas. Este mecanismo parece ser fundamental y puede explicar los nematoquistes que aparecen en el hombre cuando se evacuan rápida y casi totalmente focos pulmonares esfacelados, como sucede en la neumonía estafilocócica. En los conejos, las cavernas infladas alcanzaron su número máximo al final del segundo mes de terapéutica; a partir de ahí, hubo desinflación irregular y aplastamiento para formar pequeñas estructuras irregulares que circundaban residuos de materias esfaceladas, aunque a los dos años de terminar la quimioterapia se encontró todavía un neumatoquiste aplastado parcialmente.

Resume

L'évolution des cavités bulleuses dans la tuberculose pulmonaire expérimentale traitée d'une façon adéquate

On a démontré que des pneumatokystes géants qui apparaissent d'une façon explosive puis disparaissent, au cours d'une chimiothérapie intensive de tuberculose pulmonaire chez le lapin sont, en fait, des foyers nécrotiques, excavés et soufflés. L'excavation commence tôt et des cavernes soufflées existent déjà entre les seconde et troisième semaines de chimiothérapie. Des clapets anatomiques dans les parois des cavernes soufflées peuvent être responsables de l'air retenu, peut-être accentué par la pression sur les bronches, entre les cavités. Ce mécanisme semblerait être à la base et peut être responsable des pneumatokystes qui se produisent chez l'homme quand des foyers pulmonaires nécrotiques sont rapidement et presque complètement évacués, comme dans la pneumonie à staphyloccoque. Chez les lapins, les cavernes soufflées atteignent leur nombre maximum à la fin du second mois de traitement; après ceci, il y eut un dégonflement irrégulier et un collapsus dans les petites formations irrégulières entourant les résidus de matériaux nécrotiques, bien qu'un pneumatokyste partiellement collabé persista encore deux ans après la fin de la chimiothérapie.

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LONG-TERM CONSEQUENCES OF ISONIAZID ALONE AS INITIAL THERAPY[1]

UNITED STATES PUBLIC HEALTH SERVICE TUBERCULOSIS THERAPY TRIALS

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Introduction

Several circumstances have combined to renew interest in the effectiveness of isoniazid when used alone in the treatment of tuberculosis. Isoniazid plus para-aminosalicylic acid (PAS) is the most commonly prescribed of the chemotherapeutic regimens, but there is growing suspicion, supported by some evidence, that not all patients take their PAS (1). Moreover, many hospitals report that an increasing proportion of newly admitted patients have received isoniazid alone from private physicians before being sent to the hospital. It seems likely that many others who never reach the hospitals are similarly treated. Finally, with the increasing practice of using isoniazid for both infection and disease prophylaxis, some persons will inevitably continue to receive isoniazid alone in the interval between the development of clinical disease and its detection. The probability that many patients are being treated with isoniazid alone has prompted the United States Public Health Service to examine its material on the long-term consequences of the initial treatment of pulmonary tuberculosis with isoniazid.

In 1952, the Public Health Service, in cooperation with 22 tuberculosis hospitals, began a comparative study of three regimens: isoniazid (150 to 300 mg. daily); streptomycin (1 gm. twice weekly) plus isoniazid (150 to 300 mg. daily); and streptomycin (1 gm. twice weekly) plus PAS (10 to 12 gm. daily). The results of forty weeks of treatment among patients with initially streptomycin-susceptible infections showed that isoniazid alone was as effective as streptomycin–PAS and somewhat less effective than isoniazid–streptomycin (2).

Materials and Methods

Some time after completion of the trial, it was decided to examine a selected group of these patients: those who had received less than two weeks of chemotherapy and less than 100 days of hospitalization at any time prior to the trial, and to ascertain their condition five years after admission.

The characteristics of the patients randomly assigned to the three regimens initially were very similar. Of a total of 559 patients, 148 cannot be used in judging the therapeutic effects of the three regimens because they did not complete forty weeks of assigned treatment. The reasons are shown in table 1. Of the 411 patients who may be used for a five-year comparison of the three regimens, 150 patients had received isoniazid, 131 had received isoniazid–streptomycin, and 130 had received streptomycin–PAS. Comparison of the three regimens in table 2 does not disclose dissimilarities with respect to known initial characteristics except that the isoniazid regimen included more patients who had multiple cavities. There is, of course, the possibility that the unavoidable exclusion of those who failed to complete therapy has disturbed the initial comparability, but the apparent similarity of the remaining groups is reassuring.

Nearly all of the patients had been discharged by the time the follow-up study was undertaken. Each hospital tried to examine patients still

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TABLE 1

SELECTION OF PATIENTS FOR FIVE-YEAR EVALUATION

	Total	Isoniazid	Isoniazid–Streptomycin	Streptomycin–PAS
Total with less than 14 days of chemotherapy and less than 100 days of hospitalization prior to trial	559	189	185	185
Forty-week regimen not completed	148	39	54	55
Left against medical advice	113	29	43	41
Left with permission	11	4	3	4
Regimen refused or changed in error	7	3	2	2
Regimen changed for intolerance	15	3	4	8
Nontuberculous deaths	2	0	2	0
Available for long-term evaluation	411	150	131	130

TABLE 2

INITIAL CHARACTERISTICS OF 411 PATIENTS SELECTED FOR FOLLOW-UP STUDY

Characteristics	Isoniazid	Isoniazid–Streptomycin	Streptomycin–PAS
Number of Patients	150	131	130
	per cent		
Race and sex			
Male white	34	39	40
Male nonwhite	23	15	13
Female white	25	27	27
Female nonwhite	18	19	20
Age (in years)			
Less than 25	19	24	21
25–44	48	42	39
45 and older	33	34	40
Hospitalization prior to admission to trial			
0–30 days	79	85	80
31–60 days	17	11	15
61–100 days	4	4	5
Examination for tubercle bacilli			
Negative	21	24	19
Positive	79	76	81
Stage of disease			
Minimal	9	11	9
Moderate	43	47	46
Far advanced	48	42	45
Cavitation			
None	30	44	37
Single	13	14	12
Multiple	57	42	51

living nearby and, for the others, a variety of arrangements was made with other hospitals, the Veterans Administration, local health departments, and private physicians.

A complete follow-up study included a roentgenogram, bacteriologic examination, and history of hospitalization, surgery, and employability during the five-year interval. In spite of the mobility of the population, the multiplicity of agencies involved and the lack of adequate roentgenographic and laboratory facilities in outlying areas, some information was obtained on nearly all of the patients selected for follow-up observation as shown in table 3.

Definitive information was obtained for 385 patients (93.6 per cent). Thirty-six patients (8.8 per cent) had died, 18 from tuberculosis and 18 from other causes. Of 349 patients (84.8 per cent) who were examined, 251 had roentgenographic and sputum culture examinations, 94 had only roentgenograms, and 4 had only sputum cultures. Twenty patients (4.8 per cent) were traced even though they could not, or would not, be examined. Only 6 patients were not located, but a search of vital statistics records disclosed that they had not died in the states of their last known residence under their own names.

RESULTS

A comparison of the patients on the three regimens at the end of forty weeks of treatment provides a convenient point of departure for a consideration of the findings on the five-year examinations. Earlier reports from this trial included all patients with streptomycin-susceptible infections at the start of the trial, a number of whom had had previous streptomycin treatment. With hindsight, it can be seen that the inclusion of those who had had previous streptomycin treatment penalized the streptomycin-containing regimens. Consequently, the forty-week results in the follow-up population, from which these patients have been excluded, are somewhat more favorable for isoniazid–streptomycin and streptomycin-PAS than those reported earlier.

As shown in table 4, tubercle bacilli could not be isolated by culture after the thirty-second

TABLE 3

COMPLETENESS OF FIVE-YEAR FOLLOW-UP STUDY

	Total		Isoniazid		Isoniazid–Streptomycin		Streptomycin–PAS	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total	411	100.0	150	100.0	131	100.0	130	100.0
Located	405	98.5	147	98.0	129	98.5	129	99.2
Dead	36	8.8	15	10.0	10	7.6	11	8.5
Tuberculosis	18	4.4	11	7.3	3	2.3	4	3.1
Other causes	18	4.4	4	2.7	7	5.3	7	5.4
Examined	349	84.8	126	84.0	112	85.6	111	85.3
Not examined	20	4.9	6	4.0	7	5.3	7	5.4
Not located	6	1.5	3	2.0	2	1.5	1	0.8

TABLE 4

RESULTS OF FORTY WEEKS OF ANTIMICROBIAL THERAPY IN PREVIOUSLY UNTREATED PATIENTS

Condition at 40 Weeks		Number			Per Cent		
		Isoniazid	Isoniazid–Streptomycin	Streptomycin–PAS	Isoniazid	Isoniazid–Streptomycin	Streptomycin–PAS
Total		150	131	130	100.0	100.0	100.0
Tuberculosis deaths		5	0	2	3.3	0.0	1.5
Regimen changed because of critical worsening		5	0	5	3.3	0.0	3.8
Bacteriologic status	Roentgenographic improvement						
Culture positive for *M. tuberculosis*	None	9	2	8	6.0	1.5	6.2
	Moderate	19	6	7	12.7	4.6	5.4
	Marked	11	5	5	7.3	3.8	3.8
Culture negative for *M. tuberculosis**	None	24	20	24	16.0	15.3	18.5
	Moderate	39	45	40	26.1	34.4	30.8
	Marked	38	53	39	25.3	40.4	30.0

* No positive cultures after thirty-second week

week from the sputum specimens of 90 per cent of the patients on isoniazid–streptomycin, 79 per cent of those on streptomycin–PAS, and 67 per cent of those on isoniazid alone. From the table, the proportion of patients showing marked or moderate improvement on the forty-week roentgenograms may also be computed: 83 per cent on isoniazid–streptomycin, 70 per cent on streptomycin–PAS, and 71 per cent on isoniazid alone.

For examination of their condition at five years, patients have been classified as "bacteriologic failures" and "bacteriologic successes" after forty weeks of treatment. "Failures" include those still infectious, those whose regimen was changed during the forty weeks because of critical worsening, and those who died of tuberculosis during treatment. By this definition, the "failures" number 49 on isoniazid alone, 13 on isoniazid–streptomycin, and 27 on streptomycin–PAS. Patients whose sputum did not yield tubercle bacilli on culture after the thirty-second week have been considered "successes": 101 on isoniazid, 118 on isoniazid–streptomycin, and 103 on streptomycin–PAS.

Five-year status of forty-week "failures": After the forty-week control period, each participating physician was free to use whatever chemotherapy he wished. He had, of course, already changed chemotherapy for those who had become criti-

cally worse during the forty weeks. Among the remaining patients still infectious after forty weeks, additional chemotherapy was prescribed immediately for 87 per cent of those receiving isoniazid, 85 per cent of those receiving isoniazid–streptomycin, and 80 per cent of those receiving streptomycin-PAS. There were undoubtedly other changes later, and probably all patients still infectious after forty weeks of treatment on their original regimen were subsequently treated with other antimicrobial agents. Reliable histories for all patients could not be collected on chemotherapy prescribed during the interval between the fortieth week and the five-year follow-up observation period, since few patients were under the direct supervision of the same hospital for the entire period.

In table 5, the patients on each regimen who

TABLE 5

CONDITION AT FIVE YEARS OF PATIENTS WHO WERE "BACTERIOLOGIC FAILURES" AFTER FORTY WEEKS OF CONTROLLED THERAPY

Regimen	Total	Dead		In Hospital	Not in Hospital			
					Active Disease		Inactive Disease‡	Activity Unknown
		Nontuberculous Causes	Tuberculosis*		Culture Positive for *M. tuberculosis*	On Roentgenograms†		
Isoniazid	49	2	10	9	2	6	17	3
Isoniazid–streptomycin	13	1	—	3	—	1	6	2
Streptomycin–PAS	27	1	3	6	1	3	12	1

* Includes 5 patients receiving isoniazid and 2, streptomycin-PAS who died before the fortieth week.

† Cultures for tubercle bacilli were negative for 4, 1, and 1 patients and unavailable for 2, 0, and 2 patients receiving isoniazid, isoniazid–streptomycin, and streptomycin–PAS, respectively.

‡ Cultures for tubercle bacilli were negative for 12, 4, and 5 patients and unavailable for 5, 2, and 7 patients receiving isoniazid, isoniazid–streptomycin, and streptomycin–PAS, respectively. Roentgenograms for all were read as inactive.

TABLE 6

CONDITION AT FIVE YEARS OF PATIENTS WHO WERE "BACTERIOLOGIC SUCCESSES" AFTER FORTY WEEKS OF CONTROLLED THERAPY

Regimen	Total	Dead		In Hospital	Not in Hospital			
					Active Disease		Inactive Disease†	Activity Unknown
		Nontuberculous Causes	Tuberculosis		Culture Positive for *M. tuberculosis*	On Roentgenograms*		
	Number							
Isoniazid	101	2	1	2	1	14	75	6
Isoniazid–streptomycin	118	6	3	3	3	10	86	7
Streptomycin–PAS	103	6	1	3	1	8	77	7
	Per Cent							
Isoniazid	100.0	2.0	1.0	2.0	1.0	13.9	74.2	5.9
Isoniazid–streptomycin	100.0	5.1	2.5	2.5	2.5	8.5	73.0	5.9
Streptomycin–PAS	100.0	5.8	1.0	2.9	1.0	7.8	74.7	6.8

* Cultures for tubercle bacilli were negative for 13, 8, and 8 patients and not available for 1, 2, and 0 patients receiving isoniazid, isoniazid–streptomycin, and streptomycin–PAS, respectively.

† Cultures for tubercle bacilli were negative for 50, 60, and 55 patients and unavailable for 25, 26, and 22 patients receiving isoniazid, isoniazid–streptomycin, and streptomycin–PAS, respectively. Roentgenograms for all were read as inactive.

were "bacteriologic failures" at forty weeks have been classified according to their condition at five years. The classification has been complicated because the amount of information obtained ranged from a complete physical examination to knowledge only that the patient was not in a tuberculosis hospital and had not died.

Comparisons of the three groups at five years are limited by the small number of forty-week "failures." However, including deaths during controlled therapy, one-fifth of the isoniazid "failures" had died of tuberculosis by the end of the fifth year compared with one-tenth of the streptomycin-PAS "failures" and no deaths among the isoniazid–streptomycin "failures." The five-year fate of forty-week "failures" is of historical interest rather than practical importance, since, on the basis of experience acquired since 1952, no "failure" would today be treated with a single drug or a single combination of drugs for as long as forty weeks.

Five-year status of forty-week "successes": What happened to the forty-week "successes" is similarly shown in table 6. Among patients whose infectiousness had been reversed during the forty weeks of controlled therapy, additions in treatment reported at the fortieth week were understandably less frequent: 14 per cent changed from isoniazid alone, 8 per cent from isoniazid–streptomycin, and 3 per cent from streptomycin-PAS. Further changes were undoubtedly made between the fortieth week and the end of the five-year period.

Most patients who were "forty-week successes" were in very good condition at five years. Deaths from tuberculosis were uncommon on all regimens; only a few patients were hospitalized; and most patients had inactive disease. There is no indication that those who "succeeded" on isoniazid alone in the first forty weeks did any less well than patients who received combined therapy.

Discussion

When isoniazid's effectiveness was first reported early in 1952, 1 gm. of streptomycin twice a week plus 10 to 12 gm. of PAS daily were widely established as the chemotherapy of tuberculosis. In April, 1952, the U. S. Public Health Service, in cooperation with 22 tuberculosis hospitals throughout the country, initiated a controlled investigation to test the new drug, alone and in combination with streptomycin, in comparison with streptomycin–PAS.

Within a few months, the wisdom of using isoniazid alone was vigorously challenged (3). Isoniazid-resistant strains of tubercle bacilli could be produced in a single step *in vitro* and could be isolated from patients early in therapy. Impressed by these findings, some workers predicted frequent treatment failures if isoniazid were used alone, while others feared the widespread appearance of isoniazid-resistant strains. They strongly recommended that isoniazid be administered only in combination with other agents, and viewed even the prospect of extensive tests of isoniazid alone as "nothing short of catastrophic."

Influenced by this opinion, many investigators discontinued work on the use of isoniazid alone. The Public Health Service investigative group, on the other hand, maintained that this summary dismissal of isoniazid alone increased, rather than diminished, the need to obtain controlled observations either to confirm or deny the dire prediction. In 1952, this group and that at New York Hospital–Cornell, headed by McDermott and Muschenheim, were almost alone in this country in continuing to study isoniazid as single-drug therapy.

During the eight years that followed, physicians have used three arguments against treatment with isoniazid alone: the knowledge that fewer patients show immediate therapeutic benefit than with combined therapy; the fear of emergence of isoniazid-resistant organisms; and the expectation that frequent bacteriologic and roentgenographic relapses would be its long-term consequences. But, it is time to re-examine the available evidence to determine whether these arguments are valid, and, if so, sufficient to preclude the use of isoniazid as a single agent.

Soon after the introduction of effective antituberculous agents, it was learned that combined therapy was better than any single drug then known and streptomycin-PAS was used in all cases. When isoniazid became available, it was soon evident that it was by far the most potent single drug. However, it was only natural to consider its use primarily in combination with other agents, and in recent years isoniazid-PAS has become widely accepted as the therapy of choice.

As new drugs were introduced, they were tried alone and in combination with other agents,

particularly in the treatment of patients with more extensive disease. From one trial, a sequence of two pairs of drugs, streptomycin-pyrazinamide followed by isoniazid-PAS, was found to be more effective than isoniazid-PAS for patients with far advanced cavitary disease (4). This trial was a sequel to an earlier one (5), in which switching of antimicrobial regimens was found to be advantageous. These findings indicated that, for some patients, the simple combination of isoniazid-PAS is inadequate.

On the other hand, some investigators have maintained that for patients with less extensive disease isoniazid-PAS may be more than adequate; may, in fact, be overtreatment. An indication that this might be the case appeared in an early report of observations from the first Public Health Service trial of isoniazid (6). The findings suggested that, for patients without cavities or with only one cavity, isoniazid was as effective as either isoniazid-PAS or isoniazid-streptomycin. It was, however, decidedly less effective for patients with multiple cavities.

Subsequently the Veterans Administration undertook a controlled trial designed to compare the effectiveness of isoniazid alone with isoniazid-PAS in patients with minimal and moderately advanced noncavitary disease. In this trial, reported by Phillips in 1959 (7) after a preliminary report in 1958 (8), the therapeutic results with isoniazid alone were as good as those obtained with isoniazid-PAS. Muschenheim and his co-workers also reported good results in a small group of patients treated with isoniazid alone (9). They emphasized its possible use as initial therapy with the later addition of streptomycin-PAS, if necessary.

These short-term results indicate that for some patients isoniazid alone is as effective as combined therapy; but do drug-resistant organisms emerge more frequently? Analysis of susceptibility tests on cultures from patients in the early Public Health Service Therapy Trials showed that "if tubercle bacilli are continuously discharged during prolonged chemotherapy, the organisms nearly always develop resistance to the particular agents to which they have been exposed" (10). The appearance of more patients with isoniazid-resistant organisms when this drug was used alone than when used in combination with another agent was primarily a result of a less frequent reversal of infectiousness. More patients remained infectious and, therefore, more had resistant organisms. This suggests that, if reversal of infectiousness occurred with equal frequency on all regimens, drug-resistant organisms should emerge at the same rate. This is exactly what Phillips (7) observed. In his trial, the incidence of organisms resistant to isoniazid was nearly identical on the two regimens, isoniazid alone and isoniazid-PAS.

Finally, the third argument against isoniazid alone was apprehension about its long-term consequences. Results given in the present report would indicate that, for patients whose immediate response is adequate, the five-year prognosis is equally good, whether treated initially with isoniazid alone or in combination.

Examination of all the evidence leads to the conclusion that there is no demonstrable reason for avoiding the use of isoniazid alone in the treatment of some patients with pulmonary tuberculosis. Dissenters might ask "Why not overtreat, if you will, with isoniazid-PAS? It is doing no harm and it avoids a decision whether a patient is suitable for single-drug therapy." They overlook three cogent reasons for the appropriate use of a single drug, decreased toxicity, convenience, and economy.

Summary

In a controlled trial in 22 tuberculosis hospitals in 1952 and 1953, three groups of randomly assigned patients were treated initially for forty weeks with isoniazid alone, isoniazid-streptomycin, or streptomycin-PAS. The condition of 411 patients who completed their initial course of treatment is described five years after their entrance into the trial.

After forty weeks of treatment, infectiousness had been reversed in 67 per cent of the patients receiving isoniazid, in 90 per cent of those receiving isoniazid–streptomycin, and in 79 per cent of those receiving streptomycin-PAS.

Among patients who were "bacteriologic successes" at forty weeks, nearly all were in a favorable condition at five years. There was no difference attributable to the drugs which they had received for the first forty weeks. Patients who had received isoniazid alone did as well as those who had received combined therapy. The small number of "bacteriologic failures" at forty weeks limits the analysis of their long-term experience but, in contrast to the "successes," there is some indication that patients who were still infectious after forty weeks of treatment

with isoniazid alone had a less favorable prognosis than failures on combined therapy.

Sumario

Consecuencias a Largo Plazo de la Isoniacida Sola como Terapéutica Inicial

En un ensayo fiscalizado en 22 hospitales de tuberculosis en 1952 y 1953, a tres grupos de enfermos divididos al azar se les trató inicialmente durante cuarenta semanas con isoniacida sola, isoniacida-estreptomicina o estreptomicina-PAS. Se describe ahora el estado de todos los enfermos que terminaron su serie inicial de tratamiento a los cinco años de haber entrado a formar parte del ensayo.

Al cabo de cuarenta semanas de tratamiento, se había virado la infecciosidad en 67 de los enfermos que recibieron isoniacida, 90 por ciento de los que recibieron isoniacida-estreptomicina y 79 por ciento de los que recibieron estreptomicina-PAS.

Entre los enfermos que fueron "éxitos bacteriológicos" a las cuarenta semanas, casi todos se hallaban en estado favorable a los cinco años. No había diferencia imputable a las drogas recibidas durante las primeras cuarenta semanas. Los que habían recibido isoniacida sola lo pasaron tan bien como los que recibieron una terapéutica combinada. El pequeño número de "fracasos bacteriológicos" a las cuarenta semanas limita el análisis de lo que les sucedió a largo plazo, pero, en contraposición a los "exitos," existe alguna indicación de que los que eran todavía infecciosos al cabo de cuarenta semanas de tratamiento con isoniacida sola tenían un pronóstico menos favorable que los casos de fracaso de la terapéutica combinada.

Resume

Conséquences à long terme de l'isoniazide seul comme thérapeutique initiale

Dans un essai de contrôle, dans 22 hôpitaux pour tuberculose, de 1952 à 1953, trois groupes de malades choisis au hasard furent soignés pour commencer pendant quarante semaines, par de l'isoniazide seul, de l'isoniazide-streptomycine ou de la streptomycine et du PAS. L'état des 411 malades qui finirent le début du traitement est décrit cinq ans après le début de l'essai.

Après quarante semaines de traitement, la contagiosité changea de signe dans 67% des malades recevant de l'isoniazide, dans 90% de ceux recevant de l'isoniazide-streptomycine et dans 79% de ceux recevant de la streptomycine et du PAS.

Parmi les malades qui furent "des succés bactériologiques" au bout de quarante semaines, presque tous furent dans état favorable au bout des cinq ans. On ne put pas attribuer de différence aux médicaments qu'ils reçurent pendant les quarante premières semaines. Les malades qui reçurent de l'isoniazide seulement s'en trouvèrent aussi bien que ceux qui reçurent un traitement associé. Le petit nombre "d'échecs bactériologiques" au bout de quarante semaines limite l'analyse de leur expérience à long terme mais, à l'opposé des "succés," il y a quelqu'indication que les malades qui étaient encore contagieux après quarante semaines de traitement à l'isoniazide seul, avaient un pronostic moins favorable que les "échecs" du traitement associé.

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THE RESULTS OF CHEMOTHERAPY IN SILICOTUBERCULOSIS[1]

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Introduction

The Veterans Administration Hospital in Wilkes-Barre, Pennsylvania, is situated in an anthracite mining region where coal is extracted from silica-bearing rock. This region has employed and still employs thousands of miners exposed to coal dust and significant (silicosis-producing) amounts of silica dust. In consequence, numerous patients with silicosis and its complications have been and still are admitted to this hospital.

With the incidence of pulmonary tuberculosis increased in silicosis, cases of silicotuberculosis are not unusual in the experience of the Wilkes-Barre Veterans Administration Hospital.

This paper will present the results of therapy in a small group of miners with silicotuberculosis. A comparison will also be drawn between these results and those reported in the literature, including a previous report (1) from the Wilkes-Barre Veterans Administration Hospital of an entirely different group of patients with silicotuberculosis.

Material and Methods

Patients: The patients selected for this analysis were all hard-coal miners with active silicotuberculosis who had been admitted to this hospital between July 1, 1958, and January 1, 1960, and who had received continuous chemotherapy.

The diagnosis of active silicotuberculosis was based on the occupational history, roentgenographic evidence of such disease, and sputum positive for tubercle bacilli on microscopy or culture, or both.

During the period mentioned, 20 patients with active silicotuberculosis were hospitalized at the Veterans Administration Hospital. Two of these patients were not included in this study, since one patient had received only intermittent therapy for several years before hospitalization, and the other died of a complicating bronchogenic carcinoma after he had been hospitalized for less than one month. Of the 18 patients who are the subjects of this analysis, 7 had started and had been continued on chemotherapy in another hospital before admission to the Veterans Administration Hospital, and their records and roentgenograms were obtained. All 18 patients were males and their age distribution may be seen in table 1. They had worked in the hard-coal mines between seven and fifty years. Nine patients had been so employed for twenty years or more, while the other 9 had been so engaged for less than twenty years.

Therapy: Three regimens were employed:

(*1*) Streptomycin intramuscularly, 1 gm. daily, usually for ninety days; sodium para-aminosalicylate (PAS)[2] orally, 12 gm. daily; isoniazid orally, 300 to 600 mg. daily; and pyridoxine, 25 mg. daily when 600 mg. of isoniazid were given. Ten patients received this regimen.

(*2*) Isoniazid orally, 300 to 600 mg. daily; PAS orally, 12 gm. daily; and pyridoxine, 25 mg. daily when 600 mg. of isoniazid were administered. There were 8 patients in this group.

(*3*) Cycloserine orally, 500 mg. daily, and pyrazinamide orally, 3 gm. daily, were added to the treatment of 2 patients when their sputum still remained infectious after approximately six months of therapy with streptomycin, isoniazid, PAS, and pyridoxine. In other words, these patients were eventually on a six-drug regimen, inclusive of pyridoxine, and both received all 6 drugs for at least six months.

Evaluation of therapy: This report deals only with the results of therapy in the 18 patients while they were hospitalized; no follow-up studies of the patients after discharge are included.

The results were evaluated from roentgenographic and bacteriologic data obtained here and from other institutions prior to hospitalization here. While the patients were at the Veterans Administration Hospital, chest roentgenograms were usually taken every two months, while microscopy and cultures of sputum were performed monthly.

Reversal of infectiousness was regarded as having occurred if sputum smears and cultures were negative for acid-fast bacilli for at least two successive months; and bacteriologic relapse, if the bacteriologic studies were again positive for acid-fast bacilli on microscopy or culture after they had once disappeared.

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[1] From the Medical Service, Veterans Administration Hospital, Wilkes-Barre, Pennsylvania.

[2] Patients with decompensated cor pulmonale received a PAS compound in which sodium was absent.

Observations

Extent of disease: The roentgenographic extent of the tuberculous disease of the 18 patients (16 showing cavities) is shown in table 1. Tuberculous disease of other organs or tissues was not present except in one patient. This patient had laryngeal tuberculosis, which responded well to antituberculous chemotherapy.

The nontuberculous conditions of significance found in the patients may also be seen in table 1. Ten of the 18 showed disabling chronic bronchitis and pulmonary emphysema, 5 of the 10 patients also demonstrating decompensated cor pulmonale.

Results of therapy: The results of chemotherapy are noted in table 2. It may be seen that roentgenographic improvement occurred in 11 patients, as manifested by regression of noncavitary disease. Furthermore, reversal of infectiousness was noted in 16 patients, while in one of these patients there was transient bacteriologic relapse. Cavity closure occurred in none of the 16 patients with cavities.

There were 3 deaths. One patient with cor pulmonale died of an acute myocardial infarction; although he had received chemotherapy for forty-six months, his sputum remained positive for tubercle bacilli. The other 2 patients died of nontuberculous pneumonia superimposed on chronic bronchitis and emphysema, one of them having suffered also from cor pulmonale; both patients had reversal of infectiousness. One of these patients, with bilateral cavities, had received chemotherapy for thirty-six months.

Fourteen of the 16 bacteriologic "conversions" took place after one to six months of therapy, the other 2 occurring after twelve and twenty-four months of treatment, respectively.

The sputum of 2 patients failed to become noninfectious. The 2 bacteriologic failures had far advanced silicotuberculosis with bilateral cavities; their sputum remained infectious despite the six-drug regimen, which included pyrazinamide and cycloserine. One of them was the patient who suffered with cor pulmonale and who died of an acute myocardial infarction. The other patient, with satisfactory pulmonary function, will probably be treated by surgical means.

TABLE 1

Background Data of 18 Silicotuberculous Patients

	Number of Patients
Age distribution (in years)	
30–39	4
40–49	3
50–59	5
60–69	6
Extent of silicotuberculosis	
Far advanced	16
Moderately advanced	2
Cavities	16
Nontuberculous complications	
Chronic bronchitis and emphysema	10
Cor pulmonale, chronic bronchitis, and emphysema	5
Diabetes mellitus	2
Acute myocardial infarction	1
Nontuberculous pneumonia	2

TABLE 2

Results of Therapy

	Number of Patients
Roentgenographic improvement	11
Roentgenographic deterioration	2
Roentgenogram unchanged	5
Cavity closure	0
Reversal of infectiousness	16
Bacteriologic relapse	1*
Death	3

* No change of therapy.

Discussion

The results of chemotherapy were excellent in this group of patients, particularly from a bacteriologic standpoint, the isoniazid–PAS regimen being as effective as that of isoniazid–streptomycin–PAS. However, the drug regimen of pyrazinamide and cycloserine failed to result in reversal of infectiousness of the sputum of 2 patients originally treated with isoniazid–streptomycin–PAS.

A comparison will be drawn between these results and those of others who have written on the treatment of pneumoconiosis complicated by tuberculosis. Included in this comparison will be a previous paper (1) from the Veterans Administration Hospital in Wilkes-Barre, Pennsylvania, on the treatment of an entirely different group of patients with silicotuberculosis.

In 1952, Theodos and Gordon (2) reported

unfavorable results in the treatment of 100 patients with silicotuberculosis in that chemotherapy was bacteriologically and roentgenographically ineffective. These patients were treated with streptomycin-PAS, but the dosage of these drugs and the duration of therapy were not stated. Experiencing also poor results, Cohen and Glinsky (3), in 1953, reported on the results of treating 18 patients with silicotuberculosis with streptomycin for only one year; the sputum of none of the patients showed reversal of infectiousness.

In 1958, the Veterans Administration Hospital in Wilkes-Barre (1) reported on the therapy of 25 patients with silicotuberculosis who had received isoniazid, streptomycin, and PAS, or a combination of these drugs. Once again, the chemotherapeutic results were far from encouraging: reversal of infectiousness occurred in only 6 of the 25 patients. The superiority of the present results over those of the 1958 report may be accounted for by two differences between the two groups of patients. The first difference is that streptomycin was administered daily to 10 patients of the present study, while, in the 1958 group, this drug was in many instances given twice weekly. The second difference—probably of greater importance than the first—is that many patients of the present study were younger men, in whom the chemotherapeutic agents are generally more effective than in older age groups.

The favorable results of the present analysis duplicate two recent reports in the literature. In February, 1959, Penney (4) reported on the treatment of 26 cases of silicotuberculosis with various combinations of isoniazid, streptomycin, and PAS. The results were extremely good, with reversal of infectiousness in 22 patients. In September, 1959, Ramsay and Pines (5) reported on the therapy of 25 patients with pneumoconiosis and tuberculosis: 17 with anthracotuberculosis[3] and 8 with silicotuberculosis. Nineteen of these patients had cavities. Chemotherapy consisted of various combinations of isoniazid, PAS, and streptomycin. Recommending therapy for at least two years, these writers stressed the importance of administering not only PAS and isoniazid, but also streptomycin, 1 gm. daily, at least for several months initially. The infectious status was reversed in all but one of their patients, and he had left the hospital against medical advice. Of the 19 patients with cavities, 4 showed cavity closure. As mentioned, Ramsay and Pines stressed the importance of administering streptomycin in conjunction with PAS and isoniazid for effective results. In contrast, the results of the present study indicate that isoniazid and PAS were as effective as the three-drug regimen of isoniazid–streptomycin–PAS. This point is important because, with "open-negative" cavities, it may be advisable to continue chemotherapy for more than two years.

In brief, it is evident that chemotherapy, as indicated by the present report and by the two just mentioned, favorably influences silicotuberculosis, particularly from the standpoint of bacteriologic conversion. Moreover, from these reports and those citing poor results with streptomycin alone and with streptomycin–PAS, the impression is gained that isoniazid is an essential component of any drug regimen for the effective therapy of this disease.

[3] Anthracotuberculosis is tuberculosis superimposed upon coal-workers' pneumoconiosis, which results from the inhalation of coal dust containing insignificant amounts of silica.

Summary

Eighteen patients with active extensive silicotuberculosis, 16 of whom had cavities, received chemotherapy for various periods of time; most of them had between three and twelve months of treatment. Three regimens were employed, all of which contained isoniazid.

The results of therapy in this group of patients were excellent; 16 showed reversal of infectiousness and 11 demonstrated roentgenographic improvement. Cavity closure, however, occurred in none of the 16 patients with cavities.

This paper reflects the excellent results reported in the recent literature on the chemotherapy of silicotuberculosis.

Sumario

Los Resultados de la Quimioterapia en la Silicotuberculosis

Dieciocho enfermos que tenían extensa silicotuberculosis activa, 16 de ellos con cavernas, recibieron quimioterapia durante varios períodos de tiempo, la mayoría de ellos durante tres a doce meses. Se emplearon tres regímenes, conteniendo todos ellos isoniacida.

En este grupo de sujetos, los resultados de la

terapéutica fueron excelentes, mostrando 16 viraje bacteriológico y revelando 11 mejoría radiográfica. Sin embargo, no hubo cierre de cavernas en ninguno de los 16 enfermos que las tenían.

Este trabajo refleja los excelentes resultados descritos en la reciente literatura acerca de la quimioterapia de la silicotuberculosis.

Resume

Les résultats de la chimiothérapie dans la silicotuberculose

Dix-huit malades ayant une silicotuberculose active et extensive, 16 d'entr'eux avec cavernes, reçurent une chimiothérapie de durées variables; la plupart d'entr'eux eurent de trois à douze mois de traitement. Trois types de traitement furent employés, tous contenant de l'isoniazide.

Les résultats de la thérapeutique dans ce groupe furent excellents, 16 montrant une "conversion" bactériologique et 11 montrant une amélioration radiographique. La fermeture de la caverne ne se produisit néammoins dans aucun des 16 malades ayant des cavités.

Cet article reflète les excellents résultats rapportés dans la littérature récente sur la chimiothérapie de la silicotuberculose.

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PNEUMOCONIO-TUBERCULOSIS[1, 2]

Results of Medical and Combined Medical-Surgical Therapy

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INTRODUCTION

It is well known that coal-miners' pneumoconiosis differs from silicosis by many clinical, roentgenographic, and pathologic features. The studies published these last years in the English (1), United States (2, 3), and French literature (4) have stressed the characteristics of coal-miners' pneumoconiosis. When the disease is complicated by tuberculosis, new problems arise pertaining to its diagnosis, the effect of dust in its development and its evolution, and also the medical or combined medical-surgical therapy that should be applied. The high incidence of pulmonary tuberculosis in coal-miners' pneumoconiosis emphasizes the importance of these problems: In 1957 in the coal-miners of northern France, there were 1,095 cases of pneumoconio-tuberculosis among 25,547 pneumoconiotic coal workers (4.29 per cent). With hospitals situated in a coal-mining region, an exceptional opportunity was afforded the writers to treat a great number of tuberculo-pneumoconiotic miners.

It is the purpose of the present paper to report the roentgenographic and bacteriologic results of medical and of combined medical-surgical therapy in a group of 395 pneumoconiotic patients with tuberculosis and pneumoconiosis, the diagnosis having been made by occupational history and roentgenographic and/or pathologic examinations, and with tuberculosis diagnosed by bacteriologic studies. All of the patients were treated by long-term chemotherapy and submitted to a survey of long duration. A valuable comparison was made with a group of tuberculous non-pneumoconiotic patients.

[1] From the Pneumo-Phtisiological Clinic of A. Calmette's Thoracic Hospital and the Pasteur Institute of Lille; from the Surgical Clinics of Lille; from the Sanatorium of Helfaut; and from the Medical Mining Center of Douai, France.

[2] Presented before the Medical Session, as part of *Section 5A*, at the annual meeting of the American Trudeau (now Thoracic) Society, Los Angeles, California, May 17, 1960.

MATERIALS AND METHODS

The patients of the present study had been coal miners for ten years or more. All were admitted to the hospital between January, 1952, and March, 1958; all had bacteriologic confirmation of the diagnosis of pulmonary tuberculosis in the form of several specimens of sputum or of products obtained by gastric lavage, "expectorating aerosol" (5), bronchial washings, or by transparietal needle biopsy. The specimens were positive for tubercle bacilli on microscopy, culture, or inoculation. The pulmonary infections caused by atypical mycobacteria have not been taken under consideration in this study, although their incidence is relatively high in pneumoconiotic coal miners (6). All of the patients had roentgenographic and/or pathologic evidence of pneumoconio-tuberculosis (7). The usual chemotherapeutic regimen followed by the patients consisted of the administration of three drugs for six months or one year: streptomycin (sulfate or pantothenate) was given by the intramuscular route, usually in a dosage of 1 gm. daily; sodium salt of para-aminosalicylic acid (PAS) was given intravenously in a daily dosage of 15 gm.; isoniazid was administered orally in a daily dosage of 300 to 500 mg. A double-drug regimen (isoniazid with PAS, cycloserine, viomycin, or ethionamide) was used for long-term chemotherapy. In a few cases steroid therapy (deltacortisone in oral dosages of 20 mg. daily, associated with antimicrobial drugs) was used. This was inefficient and dangerous (8).

The follow-up period after the start of chemotherapy was six months to one year in 7.6 per cent of cases; one to two years in 10.8 per cent; two to three years in 17.7 per cent; three to four years in 31.4 per cent; four to five years in 13 per cent; and five to seven years in 19.5 per cent.

While the patients were hospitalized, the results of therapy were followed by monthly chest roentgenograms with tomograms, by bacteriologic examinations, and by monthly studies of the susceptibility of the tubercle bacilli to the antituberculous drugs. Bronchial investigations (bronchoscopy and/or bronchography) and pulmonary function tests were performed on almost all patients.

After discharge from the hospital, the patients were referred to community health agencies for continuation of observation and chemotherapy. The course of these patients was followed through the outpatient service at the hospital, where new

roentgenograms were obtained and bacteriologic examinations were performed every three months.

The therapeutic efficacy of the medical and combined medical-surgical therapy was based on the criteria of closure or eradication of cavity proved by roentgenography and stratigraphy and the reversal of infectiousness. Reversal of infectiousness was considered to have occurred when direct examination, culture, and inoculation were found to be negative for tubercle bacilli for a period of at least six consecutive months. A distinction has been made between healing (closure of cavity and reversal of infectiousness) and improvement (reversal of infectiousness only). All other cases have been considered as therapeutic failures.

TABLE 1

DISTRIBUTION BY ROENTGENOGRAPHIC FEATURES OF THE CHEST OF 277 PATIENTS WITH PNEUMOCONIO-TUBERCULOSIS AT THE BEGINNING OF CHEMOTHERAPY

	Noncavitary Tuberculosis	Cavitary Tuberculosis
Pneumoconiosis with discrete opacities	68	119
Pneumoconiosis with massive shadows	23	67

Results

Results of chemotherapy in 277 cases of pneumoconio-tuberculosis: The present study is based on 277 cases of pneumoconio-tuberculosis (table 1): 187 patients had pneumoconiosis with discrete opacities; and 90, pneumoconiosis with massive shadows; 119 patients in the first group and 67 in the second one had cavitary lesions. In other cases, there were infiltrative or conglomerate lesions without cavity.

The results of chemotherapy are summarized in table 2 and in figures 1 and 2. There were only 69 cases of cavity closure with noninfectious sputum. The percentage of good results is higher in pneumoconiosis with discrete opacities than in pneumoconiosis with massive shadows, and in limited tuberculous lesions than in far advanced tuberculosis. In 43 cases, the follow-up study (two to six years after completion of chemotherapy) has shown 7 relapses.

The results are given in detail in figure 1:

In 71 cases of pneumoconiosis with discrete opacities and noncavitary tuberculosis, healing occurred in 49.1 per cent; improvement, in 16.9 per cent; failure in 23.9 per cent; death in 10.1 per cent.

In 116 cases of pneumoconiosis with discrete opacities and cavitary tuberculosis, healing occurred in 21 per cent; improvement in 21

TABLE 2

RESULTS OF LONG-TERM CHEMOTHERAPY IN 277 CASES OF PNEUMOCONIO-TUBERCULOSIS

Extent of Pneumoconiotic Lesions	Extent and Type of Tuberculous Lesions		Immediate Results			Relapse
			Healing	Improvement	Failure	
Pneumoconiosis with discrete opacities (187 cases)	Limited lesions					
	no cavity	27	10	6	11	1
	cavity	34	10	10	14	1
	Moderate lesions					
	no cavity	44	25	6	13	2
	cavity	47	15	10	22	
	Far advanced lesions					
	cavity	35		5	30	
Pneumoconiosis with massive shadows (90 cases)	Limited lesions					
	no cavity	4	3		1	
	cavity	11	1		10	
	Moderate lesions					
	no cavity	15	3	3	9	2
	cavity	32	2	9	21	1
	Far advanced lesions					
	cavity	28		6	22	

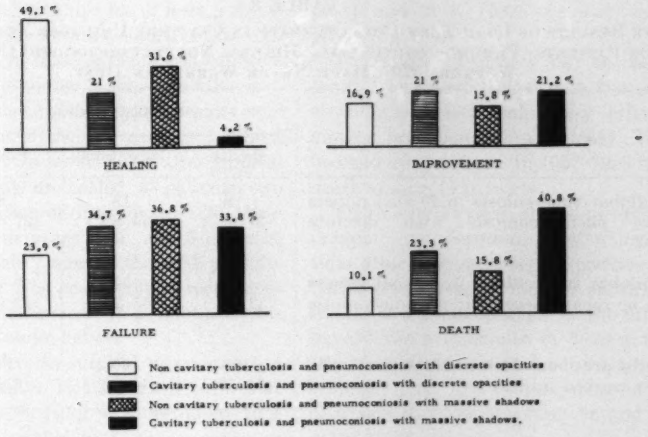

FIG. 1. Results of long-term chemotherapy in 277 cases of pneumoconio-tuberculosis.

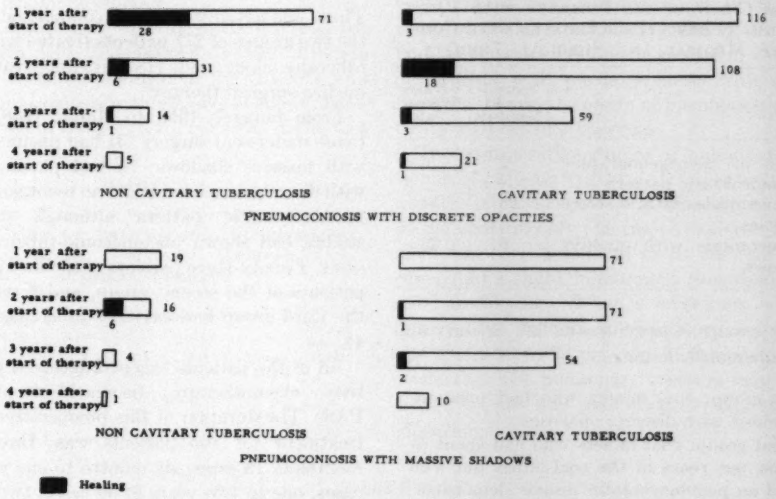

FIG. 2. Results according to the duration of chemotherapy.

per cent; failure in 34.7 per cent; death in 23.3 per cent.

In 19 cases of pneumoconiosis with massive shadows and noncavitary tuberculosis, healing occurred in 31.6 per cent; improvement in 15.8 per cent; failure in 36.8 per cent; death in 15.8 per cent.

In 71 cases of pneumoconiosis with massive shadows and cavitary tuberculosis, healing occurred in only 4.2 per cent; improvement in 21.2 per cent; failure in 33.8 per cent; death in 40.8 per cent.

The results of long-term chemotherapy are tabulated in figure 2. As may be seen, healing occurred chiefly in pneumoconiosis with discrete opacities, with a continuation of chemotherapy for one year in noncavitary lesions or for two years in cavitary lesions. There were few good results in the group of pneumoconiotic coal miners whose roentgenograms showed massive shadows.

For a better appreciation of the aggravating action of pneumoconiosis on tuberculosis, the results of the same chemotherapy were compared

TABLE 3

COMPARATIVE RESULTS OF LONG-TERM CHEMOTHERAPY IN CAVITARY UNILOBAR TUBERCULOSIS AMONG 3 GROUPS OF PATIENTS: PNEUMOCONIOTIC COAL MINERS, NONPNEUMOCONIOTIC COAL MINERS, AND WORKERS WHO HAVE NEVER WORKED IN DUST

	Immediate Results			Relapse
	Healing	Improvement	Failure	
Cavitary unilobar tuberculosis in 29 coal miners who have pneumoconiosis with discrete opacities	8 (27.6%)	13 (44.8%)	8 (27.6%)	
Cavitary unilobar tuberculosis in 31 coal miners who have no roentgenographic pneumoconiotic pattern	10 (32.2%)	14 (45.2%)	7 (22.6%)	
Cavitary unilobar tuberculosis in 212 patients who have never worked in dust	158 (74.5%)	40 (18.9%)	14 (6.6%)	7 (3.3%)

TABLE 4

DISTRIBUTION BY ROENTGENOGRAPHIC FEATURES OF THE CHEST OF 118 PATIENTS WITH PNEUMOCONIO-TUBERCULOSIS TREATED WITH COMBINED MEDICAL AND SURGICAL THERAPY

	Noncavitary Tuberculosis	Cavitary Tuberculosis
Absence of roentgenographic pneumoconiotic pattern	2	6
Pneumoconiosis with discrete opacities	25	54
Pneumoconiosis with massive shadows	8	23

on three groups of patients who had cavitary unilobar tuberculous lesions:

First group: coal miners who had pneumoconiosis with discrete opacities.

Second group: coal miners who had spent at least ten years in the coal-mines but who had no pneumoconiotic lesions demonstrable roentgenographically.

Third group: Patients who were not miners.

The results are listed in table 3. Healing occurred in 8 of 29 cases of the first group (27.6 per cent); in 10 of 31 cases in the second group (32.2 per cent); and in 158 of 212 cases in the third group (74.5 per cent).

Briefly stated, the percentage of healing for comparable tuberculous lesions is only half as great in coal miners as in patients who have never worked in a coal mine.

Results of combined medical-surgical therapy: The failure of medical therapy in pneumoconiotuberculosis justifies surgery in selected cases. There was not any available comparison between the two groups of 277 patients treated with chemotherapy alone and 118 patients treated with medico-surgical therapy.

From January, 1952, to March, 1958, 118 patients underwent surgery: 31 had pneumoconiosis with massive shadows; 79 had pneumoconiosis with discrete opacities; 8 had no roentgenographic pneumoconiotic pattern although pathologic studies had shown pneumoconio-tuberculous lesions. Twenty-three patients of the first group, 54 patients of the second group, and 6 patients of the third group had cavitary tuberculosis (table 4).

All of the patients except 2 received preoperative chemotherapy (isoniazid–streptomycin–PAS). The duration of this preoperative medical treatment for 105 patients was: three to six months in 19 cases; six months to one year in 35 cases; one to two years in 36 cases; two to three years in 15 cases. Improvement was noted in 17 cases, failure in 78 cases, and deterioration in 10 cases. Pulmonary function was normal or slightly impaired in 90 per cent of the cases.

Just prior to surgery, 25 patients were in the age group twenty-two to twenty-nine, 45 in the age group thirty to thirty-nine, and 40 in the age group forty to sixty years. All had spent many years in the coal mines: ten to twenty years in 42 cases, twenty to twenty-five years in 19 cases, and twenty-five to thirty years in 20 cases.

After completion of the surgical procedures.

chemotherapy was continued for at least a three-month period, usually for a six-month or one-year period. The course of these patients was followed. The duration of the survey is shown in table 5.

Immediately following surgery, the results were good in 116 cases. Of the 106 patients followed regularly from two to seven years after surgical resection, 81 patients are healed: 33 patients, two years after completion of drug therapy; 26, three years after; 14, four years after; and 5 patients, six years after. Thirty-nine of these 81 patients went back to work. The postoperative roentgenograms of 13 patients have shown a slight accentuation of pneumoconiotic lesions.

The results of diverse surgical procedures are summarized in table 6. Healing occurred in 6 of 7 segmentectomies; in 70 of 91 lobectomies; in 4 of 13 lobectomies, added to segmentectomy or pleurectomy; in 2 of 8 bilobectomies; and in only one of 5 pneumonectomies.

These results were superior in the group of patients who had pneumoconiosis with discrete opacities to those obtained in the patients who had pneumoconiosis with massive shadows: healing occurred in 58 cases (77.3 per cent) in the first group and in 17 (58.6 per cent) in the second group (figure 3).

The failures of surgery may be classified as follows: There were 6 deaths in the postoperative six-month period (pulmonary infarcts, pleural and/or bronchial complications). They become less and less frequent. In 1957–1958 there was one death among 49 patients.

There were 11 cases of postoperative roentgenographic deterioration. Bacteriologic examinations after surgery remained positive for tubercle bacilli on microscopy and/or culture in 8 cases. Studies of the susceptibility of the tubercle bacilli have shown a resistance in 3 cases: resistance to 10γ of isoniazid occurred in one case; to 0.5γ of isoniazid and 10γ of streptomycin in another case; and to 1γ of streptomycin and 20γ of PAS in the third case.

There were 14 incidences of relapse (14.6 per cent). These were homolateral in 12 cases and occurred in the postoperative two- or three-year period. The rate of relapse is greater in the group of patients with pneumoconiosis with massive shadows than in the group of pneumoconiotic patients with discrete opacities.

The postoperative studies of pulmonary function in 57 patients with healed disease have shown a severe impairment of vital capacity (more than 50 per cent) in only one case; a moderate impairment has been noted (30 to 50 per cent) in 18 cases; and a slight impairment (less than 30 per cent) in 38 cases. When a lobectomy was performed, the functional respiratory amputation was about 20 to 25 per cent of the preoperative coefficient. The pulmonary function may be improved by pre- and postoperative respiratory re-education.

TABLE 5

PERIOD OF FOLLOW-UP STUDY AFTER SURGERY IN 118 CASES OF PNEUMOCONIO-TUBERCULOSIS TREATED WITH COMBINED MEDICAL AND SURGICAL THERAPY

Death in the Postoperative Six-Month Period	Period of Follow-up Study				No Follow-up Study
	2–3 Years	3–4 Years	4–5 Years	5 Years or More	
6	54	30	14	8	6

TABLE 6

RESULTS OF COMBINED MEDICAL AND SURGICAL THERAPY IN 118 CASES OF COAL-MINERS' TUBERCULOSIS

		Death in the Postoperative Six-Month Period	No Follow-up Study	Follow-up Study			
				Death	Deterioration	Healing	Relapse
Segmentectomy	7	1				*6*	
Lobectomy	91	2	2	1	4	*70*	12
Lobectomy and segmentectomy	4	2	1			*1*	
Lobectomy and pleurectomy	1					*1*	
Bilobectomy	8	1	1	2		*2*	2
Pneumonectomy	5		1	2	1	*1*	
Bilateral resection	2		1		1		

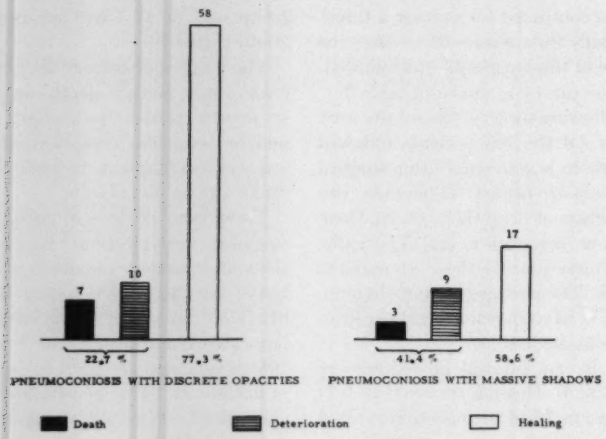

FIG. 3. Results of combined medical and surgical therapy in 104 pneumoconiotic coal miners with tuberculosis.

DISCUSSION

Briefly stated, the present paper is a report of the roentgenographic and bacteriologic effects of therapy in a group of 395 tuberculo-pneumoconiotic coal miners.

The relatively poor results of long-term chemotherapy in the patients of the present report may be explained by the fact that therapy was directed at pneumoconio-tuberculous lesions in which closure of cavity and penetration of antimicrobial drugs were impeded by fibrosis, vascular lesions, and loss of pulmonary elasticity. A prolonged dust exposition even without roentgenographic pneumoconiotic opacities is an aggravating factor for the prognosis of coal-miners' tuberculosis.

The results obtained with chemotherapy by other writers (7, 9–18) are more or less satisfactory than those of this report but, from the standpoint of cavity closure and reversal of infectiousness, they are far inferior to the results of medical therapy in non-pneumoconiotic tuberculosis.

The relative failure of chemotherapy justifies combined medical-surgical therapy when the extent of the disease and functional respiratory value allow it.

The first surgical resections in pneumoconio-tuberculosis were realized by Bonniot (19). Other writers have reported successful resectional procedure in such patients (7, 13, 18, 20–23).

The present results show that, without cavity closure after chemotherapy for six months, thoracic surgery, if feasible, should be carried out soon after the six-month period. Tuberculous lesions should be localized; the functional respiratory value should be satisfactory; vital capacity, equal or superior to 2.5 liters; maximal expiratory breathing capacity per second should be equal or superior to 1.2 liters; the oxymetry and the electrocardiogram should be normal.

The best results are obtained on patients less than forty years of age who have pneumoconiosis with discrete opacities and tuberculous lesions with tubercle bacilli susceptible to antimicrobial drugs. Lobectomy is the best surgical procedure. After surgery, chemotherapy should be continued for a six-month or one-year period.

These considerations stress the importance of dust-control measures to prevent pneumoconiosis and the necessity of early recognition of tuberculous infection. A high percentage of conglomeration is thought to be of tuberculous nature; chemotherapy should be instituted alone or combined with a surgical resection.

SUMMARY

The present study is based on a survey of long duration of 395 cases of pneumoconio-tuberculosis in coal miners.

Of a group of 277 patients treated with long-term chemotherapy, only 69 healed. Of these, 43 patients were last seen two to six years after completion of drug therapy; there were 7 relapses.

The results of chemotherapy for tuberculous coal miners were poor compared with those for non-coal-mining tuberculous patients. This bad prognosis was already evident when the fibroconiotic lesions were roentgenographically latent.

The results of chemotherapy and surgery in 118 patients were good. The lesions of 81 of a group of 106 patients who were last seen two to seven years after surgery healed. These results justify surgical therapy after preoperative six-month chemotherapy without roentgenographic improvement and when the functional respiratory value and the extent of the tuberculous lesions make it possible.

Pre- and postoperative functional respiratory re-education and postoperative chemotherapy are necessary. The gravity of pneumoconio-tuberculosis stresses the importance of early diagnosis and treatment of conglomerations.

Sumario

Tuberculosis Neumocónica. Resultados de la Terapéutica Médica y de la Médico-Quirúrgica Combinada

El estudio actual se basa en un examen prolongado de 395 casos de tuberculosis neumocónica en mineros de carbón.

De un grupo de 277 enfermos tratados con quimioterapia a largo plazo, sólo curaron 69. De éstos, a 43 se les observó por última vez de dos a seis años después de terminar la fármacoterapia; hubo 7 recidivas. Los resultados de la quimioterapia en los mineros de carbón tuberculosos fueron malos, comparados con los observados en los enfermos que tenían tuberculosis no neumoconiótica. Este pronóstico malo ya era evidente cuando las lesiones fibroconióticas eran radiográficamente latentes. Los resultados de la quimioterapia y la cirugía en 118 enfermos fueron buenos. En un grupo de 106 enfermos observados por última vez de dos a siete años después de la cirugía, las lesiones de 81 estaban curadas. Estos resultados justifican la terapéutica quirúrgica después de una quimioterapia preoperatoria de seis meses sin mejoría radiográfica y cuando lo permiten el valor respiratorio funcional y la extensión de las lesiones tuberculosas.

Son necesarias la re-educación respiratoria funcional pre- y post-operatoria y la quimioterapia postoperatoria. La gravedad de la tuberculosis neumocónica recalca la importancia del diagnóstico temprano y del tratamiento de las conglomeraciones.

Resume

Pneumoconio-tuberculose. Résultats du traitement médical et chirurgical

Cette étude est basée sur 395 cas de pneumoconio-tuberculose chez des mineurs de charbon soumis à une surveillance prolongée.

Sur 277 malades traités par chimiothérapie de longue durée, on note seulement 69 guérisons. Quarante trois de ces 69 sujets ont été suivis deux à six ans après l'arrêt du traitement médical; il y eut 7 rechutes. Les résultats du traitement médical de la tuberculose des mineurs de charbon sont de loin inférieurs à ceux observés chez des sujets non mineurs présentant des lésions tuberculeuses comparables. Ce mauvais pronostic est déjà évident même quand les lésions pneumoconiotiques sont radiologiquement latentes. Les résultats du traitement médico-chirurgical chez 118 malades sont satisfaisants. Sur 106 malades suivis deux à sept ans après l'intervention, 81 demeurent guéris.

Ces résultats justifient le recours à la chirurgie d'exérèse après une chimiothérapie de 6 mois quand celle-ci n'a pas déterminé d'amélioration radiologique et quand la valeur fonctionnelle respiratoire et l'étendue des lésions tuberculeuses le permettent. La rééducation fonctionnelle respiratoire avant et après l'intervention et la chimiothérapie post-opératoire s'imposent. La gravité de la pneumoconio-tuberculose souligne l'importance du diagnostic et du traitement précoces des confluences.

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RE-TREATMENT OF PULMONARY TUBERCULOSIS[1]

Experiences with Various Combinations of Pyrazinamide, Cycloserine, and Kanamycin in Patients Excreting Tubercle Bacilli Resistant to Both Streptomycin and Isoniazid

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Introduction

The problem of the chemotherapy of patients excreting typical *Mycobacterium tuberculosis* resistant to both streptomycin and isoniazid has been increasing in importance during the past few years. Between October 1, 1954, and October 1, 1958, 171 adult patients excreting typical mammalian tubercle bacilli, resistant to both streptomycin and isoniazid, were treated for a period of six months or longer at National Jewish Hospital at Denver. In spite of the results of the *in vitro* drug-susceptibility studies, these patients were treated with isoniazid (or its derivatives) in combination with streptomycin and/or para-aminosalicylic acid (PAS). In 9 per cent of these patients, reversal of infectiousness[3] was achieved without other drugs or surgery. In 14 per cent of these patients, in addition to antimicrobial treatment, surgical treatment resulted in reversal of infectiousness; in 77 per cent, the sputum remained positive for *M. tuberculosis* despite these measures. Thus, only 23 per cent of such patients achieved reversal of infectiousness. Such results indicate that, once a drug-resistant population of tubercle bacilli has been selected, further chemotherapy with streptomycin and isoniazid has a poor chance for success.

In an effort to develop more effective treatment programs for patients excreting streptomycin- and isoniazid-resistant tubercle bacilli in sputum, several so-called "secondary" drugs have been used, either alone or in different combinations. In this connection three clinical studies should be mentioned: Moyer and Schwartz (2) achieved reversal of infectiousness in 11 of forty-three patients with chronic cavitary disease by daily administration of 3 gm. of pyrazinamide and 500 mg. of cycloserine. Birkeland and Nissen-Meyer (3) treated cases of far advanced disease with pyrazinamide alone (40 mg. per kg. of body weight a day) or with cycloserine (500 to 750 mg. a day). Their conclusion was that the combination of these two drugs was more effective than pyrazinamide alone. In another series of twenty patients, "conversion" was achieved in nine cases with 3 gm. of pyrazinamide and 500 mg. of cycloserine daily (4).

The present study was undertaken to evaluate further the efficacy of various combinations of pyrazinamide, cycloserine,[4] and kanamycin sulfate[5] in the treatment of such patients.

Materials and Methods

Between April, 1958, and May, 1960, two studies composed of 47 and 18 patients, respectively, were conducted. These patients were excreting organisms resistant to streptomycin and isoniazid.[6] The patients ranged from eighteen to sixty-six years of age; there was a fairly equal sex distribution. The mean body weight of all of the groups was about the same, and the tuberculous pulmonary involvement could be classified as either moderately or far advanced. In the first group of 47 patients, on roentgenographic evaluation, 22 had either at least one cavity greater than 5 cm. in diameter or a

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[3] Reversal of infectiousness is defined as follows: On three consecutive monthly specimens, the sputum must be negative on concentrate smear for acid-fast rods as well as negative on culture for *Mycobacterium tuberculosis*. All subsequent sputum specimens likewise must be negative on culture for *Mycobacterium tuberculosis* (1).

[4] The cycloserine used in these studies was supplied through the courtesy of Eli Lilly & Co.

[5] The kanamycin sulfate used in these studies was supplied through the courtesy of Bristol Laboratories Inc.

[6] At National Jewish Hospital, populations of tubercle bacilli are considered to be susceptible to isoniazid and streptomycin if, on direct testing on 7H-10 solid medium, less than 1 per cent of the numbers of organisms yielding colonies on the control can grow on the same culture medium containing either 0.2 γ of isoniazid per ml. or 2 γ of streptomycin per ml. (1).

"destroyed lung." During both studies the patients were assigned to their treatment groups without regard to the extent of the disease or their treatment status.

The 47 patients in the first study were divided into two smaller groups: *Group A*, patients who received kanamycin, pyrazinamide, and cycloserine; *Group B*, patients who received pyrazinamide and cycloserine.

The dosage of pyrazinamide was 0.5 gm. three times daily by mouth. The dosage of cycloserine was 250 mg. twice daily by mouth. In most cases, cycloserine and pyrazinamide therapy was continued for at least six months. However, a few patients showed clearly that no bacteriologic response was occurring and, therefore, the regimen was discontinued between three and six months after it was started. Twelve patients were treated with at least one of these drugs for ten to thirteen months.

Kanamycin sulfate, 1.0 gm. daily, was administered by the intramuscular route four to five days a week for a period averaging five months.

In the second study the 18 patients were divided into the following groups: *Group C*, patients who received cycloserine and pyrazinamide; and *Group D*, patients who received kanamycin and pyrazinamide.

The dosage of cycloserine was increased to 0.5 gm. twice daily and the pyrazinamide dosage was increased to 40 mg. per kg., not to exceed 3.0 gm., divided into three doses daily. The kanamycin therapy was continued as already outlined.

In addition, patients in both studies received isoniazid or its hydrazone derivatives in an adequate dosage (5) to prevent possible back mutation (6). All of the patients who were treated with PAS to raise the serum concentration of antimicrobially active isoniazid were incidentally excreting organisms resistant to PAS as well as to isoniazid and streptomycin.

Pyridoxine, 50 to 300 mg. daily, was given to decrease the neurotoxicity associated with isoniazid and cycloserine therapy. Sometimes meprobamate, perphenazine (Trilafon®) and chlorpromazine (Thorazine®) were used in an effort to prevent the toxic side-effects of cycloserine.

Before and during pyrazinamide therapy, liver function studies were performed, as well as determinations of blood uric acid. Pyrazinamide was usually discontinued (at least temporarily) when the serum glutamic oxalacetic transaminase was greater than 40 units, or the bromsulfalein retention at forty-five minutes exceeded 15 per cent. Asymptomatic or symptomatic elevations of blood uric acid were not an indication to stop therapy.

Cycloserine was given only under careful observation to patients who had histories of alcoholism or central nervous system involvement (psychiatric or organic disturbance).

Kanamycin was given with caution to any patient who had a prior history of renal damage. Routine urine examinations and blood urea nitrogen determinations were made before and during the treatment. For audiometric studies a Maico E-2 audiometer was used. Audiometric studies were performed at intervals on most patients receiving kanamycin, especially when signs and symptoms of impaired hearing were encountered.

A chest roentgenogram was taken at least once every month, and the sputum for concentrate smear and culture for *Mycobacterium tuberculosis* was collected prior to and at intervals of two weeks during the study.

Toward the completion of the first study on the 47 patients, the importance of the serum assay studies was recognized. With the development of serum microbiologic assay determinations of kanamycin and cycloserine, an effort was made to correlate the emergence of resistance[7] to a specific antimicrobial agent with the biologically active serum concentration achieved by that drug at six hours following a standardized loading dose.

The serum concentration of microbiologically active kanamycin was determined at first as follows: On each of the two days prior to the study, 1 gm. of kanamycin was given. On the third and fourth days, 1 gm. was administered by the intramuscular route at 9:00 a.m., and 10 ml. of venous blood was aseptically collected at 3:00 p.m. The microbiologic assay for kanamycin was performed in a manner similar to that described for streptomycin (5). In this instance, the 1-gm. loading dose represented the total daily dose used.

When it became apparent that, in many instances, 1 gm. of kanamycin was not an effective daily dose, kanamycin serum concentrations were determined after a dose of 20 mg. per kg. of body weight, given either in one dose or in two equal doses three hours apart.

Cycloserine serum assay studies[8] were made in the following manner: For two days, each patient was given 0.5 mg. of cycloserine plus 300 mg. of pyridoxine and 800 mg. of meprobamate a day. The dosage of the latter two drugs was continued unchanged throughout the test period. On the third, fourth, and fifth days, 10 ml. of venous blood was aseptically collected six hours after a dose of

[7] *In vitro* kanamycin-susceptibility studies were performed by adding aseptically various concentrations of kanamycin to the 7H-10 medium before pouring the same into the quadrants of Felsen plates. Beside the control medium, the plate quadrants contained, respectively, 2, 5, and 10 γ of kanamycin per ml. Cycloserine-susceptibility studies were conducted similarly, except that the medium contained 5, 10, and 20 γ per ml. (5).

[8] In testing for cycloserine in body fluids, a modified filter paper disc-agar diffusion method was employed. The test organism was a strain of hemolytic *Staphylococcus aureus* isolated in this laboratory, and the procedure was made more sensitive by the incorporation of subinhibiting (5 γ per ml.) concentrations of cycloserine in the test medium (7).

250 mg., 500 mg., and in some cases 1,000 mg., was given.

Results

According to the bacteriologic findings, the 47 patients in the first study were separated into five subgroups (table 1). The first subgroup consisted of those who achieved reversal of infectiousness (no tubercle bacilli detected) with medical treatment alone and who, for subjective or objective reasons, did not wish to have or could not tolerate thoracic surgery.

The second subgroup consisted of those whose sputum became negative for tubercle bacilli but who had thoracic surgery before the three months necessary to document reversal of infectiousness. It might be assumed that this group could have achieved such reversal without surgery; however, the protocol of treatment suggested that, if possible, these patients receiving "secondary" drugs should be operated upon as soon as their sputum became negative for *Mycobacterium tuberculosis* on smear and culture.

The third subgroup consisted of those who did not "convert" their sputum, but the improvement evaluated by bacteriologic methods was such that it was believed that surgical resections could be performed.

Subgroups 4 and 5 represented patients who had sputum persistently positive for *M. tuberculosis* in spite of chemotherapy or chemotherapy and surgical treatment.

TABLE 1

Bacteriologic Results

	Group A*	Group B†	Per Cent
1. Reversal of infectiousness‡ with drugs only	7	10	36
2. Noninfectious sputum, with drugs only, but had prophylactic surgery	7	1	17
3. Reversal of infectiousness only after surgery	4	1	11
4. Sputum persistently positive for *M. tuberculosis* (drugs only)	3	7	21
5. Sputum persistently positive for *M. tuberculosis* (drugs and surgery)	5	2	15
Total	26	21	100

* Kanamycin/cyloserine/pyrazinamide
† Cycloserine/pyrazinamide
‡ As defined

TABLE 2

Bacteriologic Results

	Group C*	Group D†
1. Sputum noninfectious but not by reversal	1	
2. Reversal of infectiousness‡ with drugs only	6	2
3. Reversal of infectiousness only after surgery		1
4. Sputum persistently positive for *M. tuberculosis* (drugs only)	1	7
Total	8	10

* Cycloserine/pyrazinamide
† Kanamycin/pyrazinamide
‡ As defined

The number of reversals of infectiousness obtained with medical treatment alone was 17 (36 per cent). When those who had sputum negative for *M. tuberculosis* prior to "prophylactic" surgery and those whose response to medical treatment made surgical intervention possible were included, the "salvage" figure rose to 30 patients, or 64 per cent of the group studied. Twenty-one per cent of the patients evaluated failed to "convert" their sputum on drugs alone, and 15 per cent failed to achieve noninfectious sputum after both drugs and operation. All but 4 of the 47 patients showed some initial bacteriologic improvement, and, to the writers' knowledge, there have been two bacteriologic relapses. Finally, the addition of kanamycin to the therapeutic regimen did not appear to enhance the results significantly.

Eight of the 18 patients in the second study were treated with cycloserine and pyrazinamide (*Group C*, table 2). The remainder were treated with kanamycin and pyrazinamide (*Group D*). The categories into which these patients were placed were somewhat different because the follow-up period was shorter. The first and second subgroups consisted of patients who, on chemotherapy alone, had either a "negative but as yet not converted" sputum or whose sputum had become noninfectious. The third subgroup consisted of those whose sputum had become noninfectious after surgery, and the fourth subgroup of patients was limited to those who had

sputum persistently positive for *M. tuberculosis* despite chemotherapy.

Of the 8 patients in *Group C*, one patient on chemotherapy alone had a "negative but as yet not converted" sputum at the time of this evaluation, and 6 patients had noninfectious sputum. The sputum of the remaining patient had become noninfectious (as defined), but she was unable to take a sustained course of cycloserine. Shortly after the drug was discontinued, her sputum became positive for *M. tuberculosis* on both microscopy and culture; thereafter, she was listed in subgroup 4. Thus, 7 of the 8 patients in *Group C* showed clear response to the increased dosages of cycloserine and pyrazinamide.

The sputum of 2 of the patients in *Group D* became noninfectious on this kanamycin-pyrazinamide regimen, and in one patient this occurred only after resectional surgery. Seven patients had sputum persistently positive for *M. tuberculosis* despite chemotherapy. So far only 30 per cent of the patients in this group have achieved sputum "conversion."

Toxic reactions of various degrees were observed in 32 of the 65 patients (table 3). The largest number and most severe reactions were

TABLE 3

SIGNIFICANT DRUG REACTIONS
65 Patients Treated with Drugs for 3–6 Months

Drug Manifestation	Number of Patients				
	Group A	Group B	Group C	Group D	Total
Cycloserine					
Blurring of vision	1	0	0	0	1
Tremor	1	0	0	0	1
Convulsion	2	1	0	0	3
Psychosis	2	0	1	0	3
Marked jitteriness	3	3	0	0	6
Pyrazinamide					
Joint pains	0	2	2	1	5
Darkened skin	0	1	0	0	1
Marked loss of appetite	1	4	0	0	5
Biochemical determinations indicate marked hepatocellular damage	1	0	0	0	1
Kanamycin					
Marked subjective hearing loss	2	0	0	0	2
Significant audiometric changes	1	0	0	3	4
Total					32

TABLE 4

BLOOD CHEMISTRY RESULTS
65 Patients Treated for 3–6 Months with Pyrazinamide

Test	Normal	Number of Patients*			
		Group A	Group B	Group C†	Group D†
Serum bilirubin	1 mg. per 100 ml. total	1	0	0	0
Bromsulfalein	Less than 15 per 100 ml. retention	9	6	1‡	3
Serum glutamic oxalacetic transaminase	40 units	12	6	0	2
Uric acid (blood)	6 mg. per 100 ml.	23	17	8	10

* Patient had an abnormal test result at least once during the study.
† Patients in *Groups C* and *D* were not on pyrazinamide as long as those in *Groups A* and *B*.
‡ Bromsulfalein retention above 15 per cent prior to initiation of therapy.

noted to cycloserine. Two of the patients became psychotic; 6 experienced marked anxiety reactions; 2 had convulsions (one of which was associated with an episode of acute alcoholism); another had blurred vision; and the last had muscle spasms which appeared to be attributable to cycloserine. Thus far, the second study seems to show a marked decline in the incidence of reactions to cycloserine.

Elevation of the blood uric acid was noted in 56 of the 65 patients studied (table 4). In 3 patients the value was within normal limits, and in 6 the study was not done. Five of the patients treated with pyrazinamide had symptoms in their joints which were easily controlled with uricosuric agents. Significant darkening of the skin was observed in only one patient (8). Although loss of appetite was rather marked in 5 patients, the symptoms were reasonably well controlled with anticholinergic drugs. Bromsulfalein retention in excess of 15 per cent at forty-five minutes occurred in 19 of the patients. In one patient, the bromsulfalein retention was increased even prior to the study.

In the first study, 2 patients on kanamycin therapy noted loss of hearing. Audiometric studies before and after treatment were made on only 5 patients during the course of the first study, and only one person showed a significant hearing loss. During the second study all 10 patients in *Group D* had frequent audiometric studies, and 3 of the 10 demonstrated hearing loss in the higher frequencies.

Studies on the drug concentrations in the serum following a 1-gm. loading dose of kanamycin yielded the following results (table 5): The mean serum concentration of biologically active kanamycin achieved in 43 patients at six hours following a loading dose of 1 gm. was 18 γ per ml., with a standard deviation of $\pm$ 9 γ per ml. This was probably an inadequate concentration, since susceptibility studies done on the tubercle bacilli recovered from the sputum of 35 patients after four months of therapy with 1 gm. of kanamycin a day, four to five days a week, showed that 32 of the 35 patients were still excreting tubercle bacilli susceptible to 2 γ of kanamycin per ml. This implied failure to obtain the desired antimicrobial activity of the drug.

In an effort to achieve a better response, the total loading dose of kanamycin was increased to 20 mg. per kg., administered in two doses, each half being given three hours apart. The latter move was designed to reduce the peak rises in the serum concentration of kanamycin and provide a more sustained concentration.

The mean concentration in 43 patients at

TABLE 5

COMPARATIVE SERUM KANAMYCIN CONCENTRATIONS

At 3, 6, and 9 Hours Following a 10 Mg. per Kg. Dose Given at 0 and 3 Hours

Patient	3 Hours*	6 Hours	9 Hours
	γ *per ml.*	γ *per ml.*	γ *per ml.*
L. W.	10	17	<3
C. E.	16	25	6
E. C.	17	32	17
H. M.	20	20	11
J. S.	20	29	20
J. L.	20	40	16
J. B.	21	21	<5
C. C.	21	21	4
E. S.	21	27	6
H. P.	24	35	17
A. S.	25	20	17
G. C.	25	23	16
M. J.	25	25	13
H. A.	25	25	13
E. S.	25	31	11
G. S.	25	31	20
A. B.	25	32	25
L. C.	25	32	25
R. W.	25	45	
E. S.	25	45	25
J. L.	27	27	13
A. L.	27	31	21
J. R.	27	47	21
L. K.	31	39	16
C. A.	31	50	25
E. K.	32	25	17
S. A.	32	32	13
S. H.	32	32	25
C. J.	32	40	13
R. O.	32	40	20
G. R.	32	40	20
A. P.	32	35	35
J. N.	34	41	23
A. F.	35	60	18
F. K.	38	50	38
J. D.	39	44	32
C. R.	39	50	20
G. T.	41	34	18
J. P.	41	41	15
L. R.	47	35	21
A. P.	47	38	8
J. F.	47	60	21
J. H.	60	80	27
Mean	29	36	19
Standard deviation	$\pm$11	$\pm$14	$\pm$12
Standard error	$\pm$ 1.7	$\pm$ 2	$\pm$ 2

* At 0 hours all were less than 3.1 γ per ml.

TABLE 6

COMPARATIVE CYCLOSERINE SERUM CONCENTRATIONS AT SIX HOURS

Patient	250 Mg.	Loading Dose 500 Mg.	1,000 Mg.
	γ per ml.	γ per ml.	γ per ml.
L. W.	<2	7	
R. W.	6	18	26
R. B.	6	18	
C. E.	7	12	38
A. P.	7	22	
G. P.	8	12	22
M. J.	10	12	32
S. H.	10	22	26
J. O.	10	22	
L. R.	12	15	15
H. A.	12	18	46
J. S.	12	18	32
J. L.	12	26	
E. B.	12	38	
E. S.	15	18	
W. C.	15	22	
P. R.	18	22	
G. S.	18	32	
A. P.	18	32	
G. O.	18	46	
J. B.	22	22	
W. M.	22	22	
C. R.	22	38	
H. M.	26	18	26
L. P.	26	32	32
A. F.	26	32	38
P. K.	46	46	
Mean	16	24	30
Standard deviation	±9	±11	±13

three hours following the first half of the total dose of kanamycin was 29 ± 11 γ per ml. Six hours after the initial injection and three hours after the booster dose, the mean concentration was 36 ± 14 γ per ml. Finally, nine hours after the initial dose and six hours following the booster injection, the mean concentration was 19 ± 12 γ per ml.

Serum microbiologic assay studies were initially performed on 10 patients after a loading dose of cycloserine. Although the optimal cycloserine serum concentration is not known, at least 20 γ of biologically active cycloserine per ml. at six hours was selected. When 250 mg. were used as a loading dose, only one patient achieved a concentration in this range. Seven of the 10 patients, however, obtained a somewhat more adequate concentration six hours after a loading dose of 500 mg. of cycloserine.

The study was then repeated on 30 patients (table 6). The mean serum concentration of biologically active cycloserine six hours after a 250-mg. loading dose was 16 ± 9 γ per ml. After 500- and 1,000-mg. loading doses, the biologically active serum concentrations were 24 ± 11 γ per ml. and 30 ± 13 γ per ml., respectively.

DISCUSSION

Isoniazid, streptomycin, and/or PAS appear to be of little value in the chemotherapy of patients excreting *Mycobacterium tuberculosis* resistant to both streptomycin and isoniazid. With chemotherapy alone, the sputum in 9 per cent of 171 patients treated with these drugs at National Jewish Hospital at Denver became noninfectious, and an additional 14 per cent responded when surgical treatment was added to the regimen. In the first study conducted with combinations of kanamycin, cycloserine, and pyrazinamide, 17 of 47 patients (36 per cent) became noninfectious with chemotherapy alone, and the over-all salvage with drugs plus surgery was 64 per cent. This represents an almost fivefold improvement over the results obtained by re-treatment with isoniazid–streptomycin and/or PAS alone, and a threefold improvement over the results obtained with re-treatment with the same drugs plus surgery.

Further chemotherapy with streptomycin and isoniazid in patients excreting organisms resistant to these drugs appears to have a relatively poor chance for success. Resistance to streptomycin and isoniazid clinically seems to be an all-or-none phenomenon. The chances for reversal of infectiousness are not predictable even in the presence of therapy that has been proved to be effective when organisms susceptible to streptomycin and isoniazid are being excreted.

Although the so-called "secondary" drugs (pyrazinamide, cycloserine, and kanamycin) when compared with streptomycin and isoniazid were more effective in the treatment of such drug-resistant patients, the clinical response could not be predicted at the onset of therapy. Because *in vitro* and animal experiments had shown that these drugs are sterilizing agents for tubercle bacilli, failure to achieve a uniform clinical response suggested that the drugs were not delivered adequately. This impression was eventually verified when correlation of the bacteriologic and serum assay studies showed that in

almost all instances the patients were not receiving enough cycloserine or kanamycin.

On the contrary, it seemed fair to assume that the pyrazinamide was effectively delivered. This assumption was made because other studies had shown that populations of tubercle bacilli recovered from patients treated with pyrazinamide in doses comparable to those used in this study behaved as though pyrazinamide resistance had emerged (9, 10). Second, an initial but unsustained sputum response was frequently seen when only one drug was adequately delivered.

It soon became apparent that, if re-treatment therapy were to be more uniformly successful, two or more drugs must be delivered adequately. This confirmed a previous observation that, if one drug arrived in satisfactory concentration, sterilizing all of the susceptible organisms, but if the other drug or drugs were not delivered in sterilizing amounts, those bacteria which were resistant to the first drug by spontaneous mutation would persist. At first these organisms might be too rare to be found in the sputum, but given enough time the sputum would once again be positive.

With this in mind, the second study, involving 18 patients, was undertaken. The dosage of cycloserine was increased to 1 gm. a day. As an added safeguard, the dosage of pyrazinamide was increased to 40 mg. per kg., not to exceed 3.0 gm., divided into three doses daily.

At the time the second study was started, the kanamycin serum assay studies had not been correlated with the susceptibility studies performed on tubercle bacilli found in the sputum of patients treated with the chemotherapeutic agent. The kanamycin was given in the same manner as in the first study because, without monitoring serum concentrations, it was believed that a dosage greater than 1 gm. a day, four to five days a week, could not be justified in view of the potential eighth-nerve toxicity that might result. For reasons already given, the dosage of pyrazinamide in *Group D* was increased to 1 gm. three times daily.

The results in *Group C* seemed to be considerably better than in *Group D*, although the duration of treatment has been too short and the number of patients too small to draw any definite conclusions. It remains to be seen whether administering the daily dose of kanamycin in two injections, three hours apart, will enhance the results.

Probably as important as the correlation between the dosage of the drugs and the serum microbiologic assays achieved by the secondary antimicrobial agents is whether their usage will be limited by increased toxic reactions. The incidence of side reactions to cycloserine appears to vary from one study to another. Pyle and associates (11), using a dose between 1.5 and 2.5 gm. per day, had to discontinue the cycloserine in only 10 per cent of fifty patients. On the other hand, Ritchie and associates (9) observed that sixteen of the twenty-eight patients treated with a smaller dose of cycloserine developed involuntary and intention tremors, primarily involving the upper extremities. In the present study, the incidence of toxic reactions with cycloserine, 0.5 gm. a day, was 27 per cent. Despite increased dosage, toxic symptoms related to cycloserine in the revised treatment protocol have been well controlled with meprobamate, 800–1,200 mg. per day (12) and pyridoxine, 300 gm. per day (13, 14).

Marked hepatocellular damage was observed in only one of the patients, and this proved to be reversible following the discontinuation of pyrazinamide. The rise in the blood uric acid rarely appeared to be of any clinical significance and, when joint symptoms did occur, these were quickly controlled with uricosuric drugs. Although Morrissey and Rubin (15) believed that the serum glutamic oxalacetic transaminase was the most sensitive test to monitor hepatic function, it was the present writers' impression that the absolute level of the serum glutamic oxalacetic transaminase or the bromsulfalein was not so important as the continued deterioration of liver function tests and the clinical status of the patient.

Schwartz (16) recorded a 39 per cent onset of deafness in 75 patients who received a gram of kanamycin alone or with PAS or tetracycline phosphate complex. Only 5 of the 26 patients in the present *Group A* had frequent audiometric recordings, and the studies showed a significant hearing loss in one of this group. Two other patients in *Group A* showed impairment of their hearing which was detected clinically. In the second study, all of the *Group D* patients had frequent audiometric studies, and 3 of the 10 displayed impairment of their hearing in the higher frequencies. It remains to be seen whether

administration of divided dosage of kanamycin will alter the incidence of eighth-nerve toxicity. The results of these clinical experiments suggest that the combination of pyrazinamide and cycloserine might be one of the best regimens available at this time for re-treatment cases, if the doses are high enough: pyrazinamide, 40 mg. per kg. of body weight; and cycloserine, 1 gm. a day. Three hundred milligrams of pyridoxine and 800 to 1,200 mg. of meprobamate daily seem to give reasonable protection against cycloserine complications. Frequent liver function studies are a necessity during pyrazinamide treatment.

Summary

Sixty-five patients with chronic pulmonary tuberculosis excreting tubercle bacilli resistant to streptomycin and isoniazid were treated with pyrazinamide, cycloserine, and/or kanamycin. In 17 of 47 patients reversals of infectiousness occurred with chemotherapy alone when 1.5 gm. of pyrazinamide and 0.5 gm. of cycloserine were given daily with or without kanamycin (4 to 5 gm. weekly). In an additional 13 cases, chemotherapy made surgical treatment possible, which then resulted in reversal of infectiousness. These results were compared with results achieved in 171 patients with similar disease treated with isoniazid, streptomycin, and/or PAS. The "conversion" rate achieved with the experimental regimens was approximately five times higher; it was three times higher when the patients who underwent operation were included. There was no significant difference between the results whether kanamycin was used or not.

Eighteen patients were treated in the second study, and 7 of the 8 who received 1 gm. of cycloserine daily and 40 mg. of pyrazinamide per kg. (not to exceed 3 gm. daily) became noninfectious with drugs alone. Of the 10 patients who received the same dose of pyrazinamide and 5 gm. of kanamycin weekly, 2 became noninfectious with drugs alone and one with drugs and surgery.

Almost all of the patients received isoniazid to prevent a back mutation. Although the number of patients is small, it was believed that the regimen with higher doses of pyrazinamide and cycloserine was effective for the treatment of pulmonary tuberculosis when the tubercle bacilli recovered from the sputum were resistant to streptomycin and isoniazid.

Toxic reactions associated with kanamycin, cycloserine, and pyrazinamide appeared frequently, but were not severe enough to contraindicate such chemotherapy.

Sumario

Re-tratamiento de la Tuberculosis Pulmonar. Observaciones con Varias Combinaciones de Piracinamida, Cicloserina y Kanamicina en Enfermos que Excretaban Bacilos Tuberculosos Resistentes tanto a la Estreptomicina como a la Isoniacida

A 65 enfermos de tuberculosis pulmonar crónica que excretaban bacilos tuberculosos resistentes a la estreptomicina y la isoniacida se les trató con piracinamida, cicloserina y/o kanamicina. En 17 de 47 enfermos, se obtuvo viraje de la infecciosidad con la quimioterapia sola cuando se administraron diariamente 1.5 gm. de piracinamida y 0.5 gm. de cicloserina con o sin kanamicina (4 a 5 gm. semanalmente). En otros 13 casos, la quimioterapia permitió aplicar tratamiento quirúrgico, que luego resultó en viraje de la infecciosidad. Estos resultados se compararon con los logrados en 171 sujetos que padecían de enfermedad semejante y fueron tratados con isoniacida, estreptomicina y/o PAS. El coeficiente de "virajes" obstenido con el régimen experimental fué unas cinco veces mayor; fué unas tres veces mayor cuando se comprendía a los enfermos operados. No hubo mayor diferencia en los resultados ya se usara o no kanamicina.

En el segundo estudio, se trató a 18 enfermos, y 7 de los 8 que recibieron a diario 1 gm. de cicloserina y 40 mg. de piracinamida por kg. de peso (sin exceder de 3 gm. diarios) dejaron de ser infecciosos debido a las drogas solamente. De los 10 enfermos que recibieron la misma dosis de piracinamida y 5 gm. de kanamicina semanalmente, 2 dejaron de ser infecciosos con las drogas solamente y uno con las drogas y la cirujía.

Casí todos los enfermos recibieron isoniacida para impedir una retromutación. Aunque el número de enfermos es pequeño, pareció que el régimen de dosis mayores de piracinamida y cicloserina resulto eficaz para el tratamiento de la tuberculosis pulmonar cuando los bacilos tuberculosos retirados del esputo eran resistentes a la estreptomicina y la isoniacida.

Aparecieron frecuentemente reacciones tóxicas vinculadas con la kanamicina, la cicloserina y la piracinamida, pero no de tal gravedad que contraindicaran dicha quimioterapia.

Resume

Retraitement de la tuberculose pulmonaire. Expériences avec des associations variées de pyrazinamide, de cyclosérine, et de kanamycine chez des malades expectorant des bacilles tuberculeux résistant à la fois à la streptomycine et à l'isoniazide

Soixante-cinq malades ayant une tuberculose pulmonaire chronique, expectorant des bacilles tuberculeux résistant à la streptomycine et à l'isoniazide, furent traités avec de la pyrazinamide, de la cyclosérine et/ou de la kanamycine. Chez 17 des 47 malades, le "changement de signe" de la contagiosité se produisit avec la seule chimiothérapie quand on donna quotidiennement 1,5 gm de pyrazinamide et 0,5 gm de cyclosérine, avec ou sans kanamycine (4 à 5 gm par semaine). Chez 13 cas de plus, la chimiothérapie rendit le traitement chirurgical possible, ce qui aboutit au changement de signe de la contagiosité. Ces résultats furent comparés aux résultats obtenus chez 171 malades ayant une maladie semblable, traités avec de l'isoniazide, de la streptomycine, et/ou du PAS. Le taux de "conversion" atteint avec les traitements expérimentaux fut à peu près cinq fois plus élevé; il fut trois fois plus élevé quand les malades subirent une opération furent inclus. Il n'y eut pas de différence notable entre les résultats que l'on ait employé la kanamycine ou pas.

Dix-huit malades furent traités dans une seconde étude, et 7 des 8 qui reçurent 1 gm de cyclosérine par jour et 40 mg de pyrazinamide par kg (sans dépasser 3 gm par jour) devinrent non contagieux avec les médicaments seulement. Parmi les 10 malades qui reçurent la même dose de pyrazinamide et 5 gm de kanamycine par semaine, 2 devinrent non contagieux avec seulement les médicaments, et un avec médicaments plus chirurgie.

Presque tous les malades reçurent de l'isoniazide pour prévenir un retour de mutation. Bien que le nombre de malades soit petit, on croit que le traitement avec de fortes doses de pyrazinamide et de cyclosérine a été efficace dans le traitement de la tuberculose pulmonaire, quand les bacilles tuberculeux obtenus à partir de l'expectoration furent résistant à la streptomycine et à l'isoniazide.

Des réactions toxiques associées à la kanamycine, la cyclosérine et la pyrazinamide apparurent fréquemment, mais ne furent pas assez sévères pour contrindiquer une telle chimiothérapie.

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THE DETERMINATION OF THE ISONIAZID INACTIVATOR PHENOTYPE[1]

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Introduction

When isoniazid was first given to large groups of patients, it became apparent that there were differences in its metabolism between individuals which were strongly suggestive of a polymorphic system. "Polymorphism may be defined as the occurrence together in the same habitat of two or more discontinuous forms of a species in such proportions that the rarest of them cannot be maintained merely by recurrent mutation." (1).

Bönicke and Reif (2) determined the essential differences between the two phenotypes. Those they term "bad excretors" had small amounts of free isoniazid in the blood and urine following an oral dose of the drug, while "good excretors" had larger amounts in both the blood and urine following the same dosage. The amounts of changed, bacteriologically inactive isoniazid found were the converse of the free form. It was found by Hughes and her colleagues (3–5) that the differences lay largely in the degree of acetylation of the drug. Virtually all of the isoniazid and its metabolites could be recovered from the urine of all of the subjects. Later, the distribution of the percentage of the dose excreted as free isoniazid in the urine was shown to be bimodal (6, 7). These determinations were performed by chemical techniques. Similar results could be obtained by the application to the serum of a microbiologic technique (8, 9).

Recently it has been shown, using both a chemical (10) and a microbiologic (11) technique, that the isoniazid inactivator phenotype is genetically determined. "Slow" inactivators of isoniazid are autosomal homozygous recessives, while "rapid" inactivators are genetically of two types—heterozygotes and persons homozygous for the allele controlling the dominant character. In populations of European descent, the proportion of each phenotype in the population is 50 per cent. There are indications that the percentage of rapid inactivators is much greater among the Japanese (12) and the Eskimos (13).

The reliability of the microbiologic technique of isoniazid estimation in common use has been shown to be fairly satisfactory (14, 15). It has been pointed out, however, that the employment of a chemical method sensitive enough to detect the small concentrations of isoniazid in the plasma would be preferable (9).

Chemical methods fulfilling this criterion are now available (16, 17). The purpose of the present communication is to compare a slight modification of the method of Maher and associates (16) with a standard microbiologic assay technique for the purpose of determining the isoniazid inactivator phenotype.

Materials and Methods

The chemicals and apparatus employed were those described by Maher and co-workers (16). The experimental subjects were white and U. S. Negro patients at the Veterans Administration Hospital, Baltimore, Maryland.

Chemical Estimation of the Free Isoniazid Content of Plasma

Slight modifications of the method of Maher and co-workers (16) were employed. The quantity of 0.1N hydrochloric acid used was increased from 4.0 to 4.5 ml. to facilitate easy recovery of 3.0 ml. of acid layer after shaking with organic solvent. All of the specimens were read against distilled water at 365 mμ in a Beckman DU spectrophotometer. Each determination procedure comprised an array of six isoniazid "standards" (0, 1, 2, 3, 4, and 5 γ per ml. in duplicate) made up in pooled plasma, and 10 unknowns in duplicate. The isoniazid concentration in unknown samples was determined using the regression curve of optical density upon isoniazid concentration given by the standards.

By this method, "recovery" of pure isoniazid added to an unknown was practically 100 per cent. The standard error of estimation was determined as the mean value of

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Standard deviation of scatter of points about the line / Regression coefficient

for 55 arrays of standards. It was 0.224 γ of isoniazid per ml. of plasma.

Procedure of Isoniazid Inactivation Test on Human Subjects

There are two ways in which the genetic aspects of isoniazid metabolism may be studied in human subjects. First, urine may be collected for a certain period following the ingestion of a dose of isoniazid and analyzed for the free compound; the excretion of the free compound can then be expressed as a percentage of the total dose administered. Second, the plasma concentration of free isoniazid may be determined at a fixed interval of time following the ingestion of a standard dose.

Fractional urine collections are notoriously inaccurate unless conducted in a metabolic ward. This was shown in a study of isoniazid excretion by Storey and associates (1959) (18). Therefore, it was decided to rely upon the plasma concentration of isoniazid as the index of a subject's metabolism.

Estimation of Plasma Concentrations at Two Time Intervals after Isoniazid Ingestion

Ten subjects had blood drawn while they were fasting, and then were given 10 mg. of isoniazid per kg. of body weight orally with a small quantity of water. They remained in the fasting state and blood was taken at 90 minutes after isoniazid ingestion. A light meal of toast and coffee was then allowed. Blood was taken again six hours after isoniazid ingestion. Seven subjects had a 10 mg. per kg. dose, and had blood drawn at two and six hours following drug ingestion.

Plasma Isoniazid Concentrations Six Hours following Drug Ingestion

The patients hospitalized at the Veterans Administration Hospital were given 10 mg. of isoniazid per kg. of body weight orally in a single dose. A light meal of toast and coffee was allowed after the compound had been given. Two specimens of blood were withdrawn six hours after the isoniazid had been ingested. One specimen of 30 ml. of blood was withdrawn with a syringe containing 0.3 ml. of heparin to prevent coagulation. This specimen was stored at +4°C. (plasma specimens obtained in this way were delivered to the Johns Hopkins Hospital once a week). The second specimen was allowed to clot, and then the serum was separated for microbiologic assay of its isoniazid concentration.

The same patients were subjected on a different occasion to the same procedure using a dose of 4 mg. of isoniazid per kg. of body weight. Blood for microbiologic assay only was withdrawn six hours following drug ingestion.

Microbiologic Assays of Plasma Isoniazid Concentrations

These were performed by a standard microbiologic technique (14, 15) by Mrs. Fern B. Wittstadt at the Veterans Administration Hospital, Baltimore.

Statistical Techniques

Standard methods were used to compute regression, to compare regression coefficients, to perform analysis of variance tests, and to check regressions for linearity. These were taken from Brownlee (1949) (19). The formula used to compute the standard deviation of scatter of points about a regression line was that given by Cornfield (1958) (20) and is as follows:

Standard deviation of scatter =

$$\mathrm{Sr} = \sqrt{\frac{\sum (y - \bar{y})^2 - b^2 \sum (x - \bar{x})^2}{n - 2}}$$

where $\sum$ = "the sum of"
$\bar{y}$ = mean value of y
b = slope or regression coefficient
$\bar{x}$ = mean value of x
n = number of pairs of observations of x and y.

Results

Disappearance of Isoniazid from the Plasma

Estimations at hourly intervals after isoniazid ingestion: A "rapid" and a "slow" inactivator subject (whose states were determined by microbiologic assay with the six-hour plasma concentration method described below) were given 10 mg. of isoniazid per kg. of body weight orally. Blood was drawn hourly after drug ingestion. The relationship of $\log_{10}$ isoniazid concentrations to time is shown in figure 1.

The regression equations ($\log_{10}$ isoniazid concentrations y upon time x) for the two lines were as follows:

Slow inactivator $y = 1.234 - 0.099x$
$\mathrm{Sr} = 0.013$ units of y

Rapid inactivator $y = 0.999 - 0.197x$
$\mathrm{Sr} = 0.087$ units of y.

When the two regression coefficients are compared, $t = 6.581$ and with 12 degrees of freedom $P < 0.001$. This shows that there is a highly significant difference between the two slopes.

The standard deviation of scatter about the line (in units of y) is 6.7 times larger for the rapid inactivator than for the slow. This is

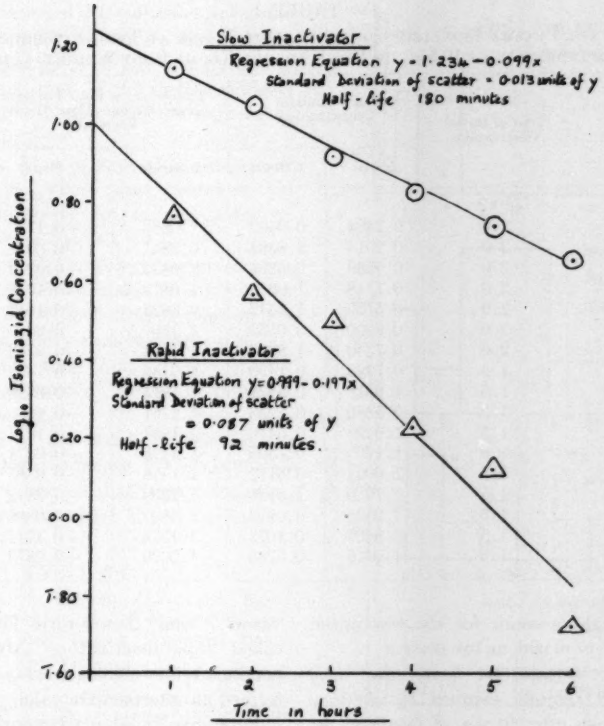

FIG. 1. Fall in plasma isoniazid concentration in representative "slow" and "rapid" inactivator subjects.

ascribed to the fact that sufficient blood was taken from the slow inactivator to perform duplicate determinations, whereas only sufficient blood for single determinations was taken from the rapid inactivator at each venipuncture.

From these regression lines the "half-life" of the isoniazid concentration in the plasma may be calculated. For the rapid inactivator this was 92 minutes, and for the slow inactivator 180 minutes.

In a different experiment, the slow inactivator subject (*DAPE*) was given 10 mg. per kg. of isoniazid orally, and blood was taken at 90 minutes and six hours thereafter. The $\log_{10}$ of the isoniazid concentrations plotted against time allowed a straight line to be drawn between these two points. The half-life of isoniazid concentration in the plasma calculated from this line was 181 minutes. It seems, therefore, that two determinations of plasma isoniazid at some hours apart will allow a fairly accurate determination of the half-life to be made.

Estimates of plasma concentrations at two time intervals after isoniazid ingestion: The half-life of isoniazid in the plasma has been computed by the two point method in 16 other subjects. The data are shown in table 1. The determinations were made by the chemical method.

The persons classified by their six-hour plasma isoniazid concentration (by the method given below) as slow inactivators have half-lives ranging from 169 to 234 minutes. Those classified by their six-hour plasma isoniazid concentration as rapid inactivators have half-lives ranging from 64 to 124 minutes. There is one exception to the last statement: subject *JEC* had a six-hour plasma concentration of 0.69 γ per ml. and a half-life of 198 minutes. This patient was under treatment for pulmonary tuberculosis but had no symptoms to suggest an alimentary absorptive

TABLE 1

EXAMINATION OF THE PLASMA ISONIAZID CONCENTRATION FOUND AT TWO DIFFERENT TIME INTERVALS FOLLOWING THE INGESTION OF 10 MG. OF ISONIAZID PER KG. OF BODY WEIGHT: CHEMICAL METHOD

Name of Subject	Time of Initial Observations	Log_{10} Isoniazid Concentration		Parameters of Log_{10} Isoniazid Concentration—Time (Hours) Regression		"Half-life" of Plasma Isoniazid Concentration
		Initial	6 Hours	Intercept	Slope	
	hours					*minutes*
FS	2.0	0.7404	0.0492	1.0868	−0.173	105
JEC	2.0	0.2014	$\bar{1}$.8388	0.3841	−0.091	198
CJJ	2.0	0.9289	0.6201	1.0833	−0.0772	234
JJM	2.0	0.7118	$\bar{1}$.004	1.0173	−0.1528	118
CG	2.0	0.5729	$\bar{1}$.9542	0.8823	−0.1547	117
GEC	2.0	0.9890	0.6532	1.1567	−0.0839	215
MW	2.0	0.7340	$\bar{1}$.8692	1.1664	−0.2162	84
JTL	1.5	0.7993	0.1430	1.0186	−0.1458	124
DAPE	1.5	1.0318	0.5821	1.1815	−0.0999	181
AS	1.5	0.9380	0.2253	1.1741	−0.158	114
KAM	1.5	1.0228	0.5416	1.1834	−0.107	169
NH	1.5	1.1201	0.7505	1.2428	−0.0821	220
MFS	1.5	1.0094	0.6117	1.1416	−0.0883	205
VC	1.5	0.7980	$\bar{1}$.5185	1.2239	−0.2842	64
EM	1.5	1.0596	0.6972	1.1803	−0.0805	224
UG	1.5	0.8802	0.1072	1.1376	−0.1717	105
Mr. P.	1.5	1.0719	0.6785	1.2029	−0.0874	207

defect which might account for the low initial concentration of isoniazid in his plasma.

Isoniazid concentrations six hours after drug ingestion in 123 subjects obtained by chemical assay of the plasma after 10 mg. of isoniazid per kg. and by microbiologic assay of the serum after 10- and 4-mg. per kg. doses: The chemical and microbiologic assays after the 10-mg. per kg. dosage were performed on blood removed at the same time from 123 patients. The 4 mg. per kg. tests were performed at another time and the assay was carried out by a microbiologic method. All of the subjects had been without medication for at least 48 hours before the tests were performed. The blood was taken six hours after the drug was ingested.

After a dose of 10 mg. of isoniazid per kg. of body weight, subjects with concentrations of 2.50 γ of isoniazid per ml. of the plasma and higher, as assessed on the chemical test, were categorized "slow inactivators," while those with values up to and including 2.49 γ per ml. were classed "rapid inactivators." From the results of the microbiologic assay technique after the 10 mg. of isoniazid per kg. of body weight dosage, subjects with concentrations of 2.40 μ were termed "intermediates," those with higher values were termed "slow inactivators," and those with lower values were called "rapid inactivators." After a dose of 4 mg. per kg., a microbiologic assay level of 0.60 was termed an intermediate value.

Forty-five rapid inactivators and 59 slow inactivators were classified the same by all three tests. In the remaining 19 subjects there was disagreement among the tests. The details of the disagreement are given in table 2.

Repeat microbiologic tests were required to classify subjects who were given an intermediate value. It may be seen that 3 subjects—*SW*, *CCE*, and *JCMcC*—fell in the intermediate class by microbiologic assays after both dosages. With the chemical method, an arbitrary division can be made at the 2.49–2.50 γ per ml. concentration. This will give rise to some degree of error, as the tails of the "slow inactivator" and "rapid inactivator" distribution curves overlap to some extent on either side of this point. The error introduced by making this arbitrary division is considered small.

The results are shown as histograms in figure 2. These histograms suggest that the definition of bimodality is clearer with the chemical results than with the microbiologic results even when the classes for the former are made as large as 1 γ per ml.

Results by the chemical determination technique: When the 128 subjects who were classified by the chemical technique were divided by ethnic group, it was found that 39 of 78 white subjects and 26 of 50 U. S. Negro subjects were rapid inactivators (table 3).

For these data, $X^2 = 0.0489$ with 1 degree of freedom $P > 0.50$. There was, therefore, no significant heterogeneity for ethnic group on these small numbers studied.

Correlation of the chemically determined concentration of free isoniazid in the plasma with the microbiologically determined concentration of free isoniazid: Plasma and serum samples were obtained at the same time from 125 subjects six hours after the ingestion of 10 mg. of isoniazid per kg. of body weight.

The regression of the chemical estimate (y) on the microbiologic estimate (x) of isoniazid concentration in γ per ml. has been calculated. The result is shown in figure 3, and indicates a strongly positive correlation. The correlation coefficient $r = 0.867$, with 123 degrees of freedom and, hence, $P < 0.001$. The regression equation is $y = 0.720 + 0.8115x$. The standard deviation of scatter of the points about the line

TABLE 2

DATA ON SUBJECTS WHOSE ISONIAZID INACTIVATOR PHENOTYPE WAS NOT SHOWN TO BE THE SAME BY THE THREE METHODS OF TESTING*

Subject	Dosage 4 Mg. of Isoniazid per Kg. of Body Weight Bioassay		Dosage 10 Mg. of Isoniazid per Kg. of Body Weight Chemical Assay		Dosage 10 Mg. of Isoniazid per Kg. of Body Weight Bioassay	
PHK	1.2	S†	2.33	R	2.4	I
FHG	1.2	S	3.34	S	2.4	I
SW	0.6	I‡	2.54	S	2.4	I
OB	0.6	I	1.22	R	2.4	I
WB	0.3	R§	1.87	R	2.4	I
ASM	2.4	S	2.45	R	2.4	I
CR	0.3	R	0.38	R	2.4	I
WEC	1.2	S	3.25	S	2.4	I
CCE	0.6	I	2.17	R	2.4	I
HAW	2.4	S	1.56	R	2.4	I
JCMcC	0.6	I	1.81	R	2.4	I
JBB	1.2	S	2.05	R	2.4	I
RFS	0.6	I	0.85	R	1.2	R
WES	0.6	I	0.92	R	1.2	R
CSH, Jr.	0.6	I	0.90	R	1.2	R
JFD	0.6	I	1.35	R	1.2	R
HG	0.6	I	1.38	R	1.2	R
EBL	0.6	I	0.68	R	0.6	R
OS	2.4	S	1.95	R	4.8	S

* Figures give plasma isoniazid concentration in γ per ml. six hours following drug ingestion.
† S—slow inactivator.
‡ I—intermediate.
§ R—rapid inactivator.

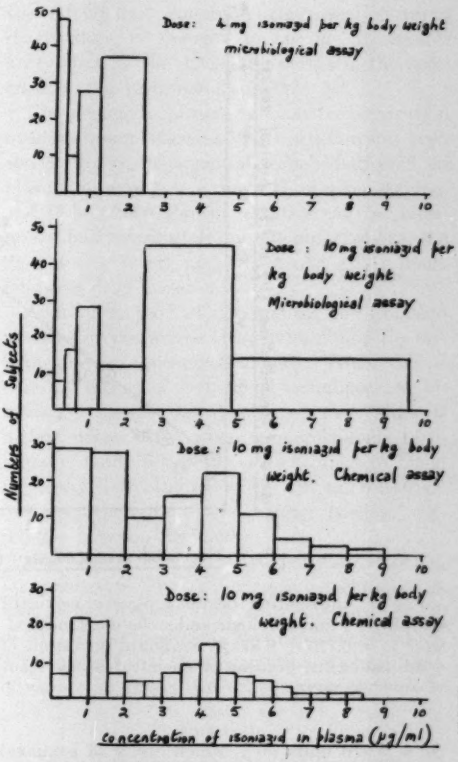
Dose: 4 mg isoniazid per kg body weight
microbiological assay
Dose: 10 mg isoniazid per kg body weight
Microbiological assay
Dose: 10 mg isoniazid per kg body weight. Chemical assay
Dose: 10 mg isoniazid per kg body weight. Chemical assay
Numbers of Subjects
Concentration of isoniazid in plasma (μg/ml)

FIG. 2. Plasma isoniazid concentrations determined by different techniques and after different dosages of the drug in 123 subjects.

TABLE 3

THE DISTRIBUTION OF THE TWO ISONIAZID INACTIVATOR PHENOTYPES

Population	Rapid Inactivator	Slow Inactivator	Totals
Tuberculous white subjects	39	39	78
Tuberculous U. S. Negro subjects	26	24	50
Totals	65	63	128

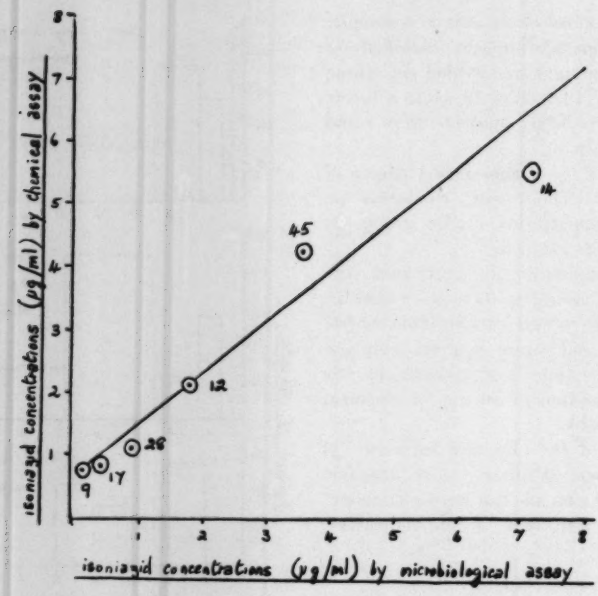

FIG. 3. Correlation between plasma isoniazid concentrations determined on the same blood sample by a microbiologic and a chemical method. Correlation coefficient r = 0.867; regression equation y = 0.720 + 0.811×. Standard deviation of scatter of points about the regression line = 0.976 γ/ml. isoniazid; ⊙ mean of chemical assay results at each microbiologic assay level, with number of observations.

Sr = 0.976 units of y, which are γ of isoniazid per ml.

The mid-class micobiologic value has been taken to calculate this regression. When the microbiologic value indicated the result to be between 0.3 and 0.6, then 0.45 γ per ml. was taken; when the result was indicated to lie between 4.8 and 9.6, then 7.2 γ per ml. was taken as the value of x.

DISCUSSION

The results of chemical and microbiologic assays performed on the same blood specimen show the former to be superior for phenotyping. Microbiologic assay methods can only give a result in terms of a discontinuous variable. From their very nature they give rise to a proportion of "intermediate" results, which can only be categorized as either "slow" or "rapid" inactivators by a repetition of the test. This proportion is usually of the order of 10 per cent of all initial tests performed.

The chemical determinations, however, express the results in terms of a continuous variable. The standard error of accuracy of this determination is approximately 0.25 γ per ml. (in the range of 0 to 5 γ per ml). When the "classes" in the histogram are made this small, then the curves can be conveniently divided at 2.50 γ per ml. This will give rise to a certain amount of misclassification, but this is probably less than 10 per cent. There are probably many factors which contribute to the presence of such large variances in the distribution curves of the six-hour plasma isoniazid concentrations. Plasma has a certain degree of nonspecific optical density (17). The variance of this optical density contributes to the total variance of both the "rapid" and "slow" inactivator curves.

There was no control over the amount of liquid ingested and excreted by the subjects. It may well be that different degrees of diuresis may produce a variance in the speed of elimination of both free and conjugated isoniazid in the urine. Some form of standardization of liquid intake and output might help to reduce the vari-

ance of the two curves. One possibility is to have all subjects in a fasting state, with nothing to drink since the previous evening, and to give them a quantity of water calculated in terms of "metabolically active mass" (21) with their dose of isoniazid. It has been shown in another series of subjects (10) that there is a significant correlation between plasma isoniazid concentration and weight in both inactivator phenotypes when the dose is calculated on a milligram per kilogram basis. The effect is more marked in the "slow inactivators" than among the "rapid inactivators." It might well be, therefore, that calculating the dose in terms of "metabolically active mass" (21) might reduce or even eliminate the variance due to this correlation. Finally, it is possible that differences in absorption may contribute to the variances. A method of circumventing this difficulty has been to use intravenous administration of the drug (22), but this limits the applicability of the procedure.

Despite the presence of a strongly positive correlation, there was a discrepancy between the values determined by the chemical and by the microbiologic assays, as is shown in figure 3. When these data were tested by the analysis of variance method, the departure from linearity was found to be significant ($P < 0.001$). At the lower concentrations, the nonspecific optical density of plasma (17) may have made the chemical result higher than the microbiologic result. At the higher concentrations, the mid-class value of 7.20 γ per ml. for the microbiologic assay method was considerably higher than the mean chemical result of 5.53 γ per ml. given by the subjects in that class.

The sample of U. S. Negro subjects studied showed the same distribution of phenotypes as did the sample of the white subjects. This finding suggests that the phenotype distribution in the U. S. Negro population is much more similar to that of Europeans than to that reported for Japanese (12) and for Eskimos (13).

Summary

It has been found that there are two distinct patterns of isoniazid metabolism present in the human species. Some persons show a rapid fall in plasma isoniazid concentration following drug ingestion, while others show only a slow fall. An individual human subject has a consistent pattern. This pattern is genetically determined; the Mendelian dominant character of rapid inactivation is thought to be due to greater acetylation of the drug than occurs in the presence of the recessive character.

The decline in plasma isoniazid concentration over six hours following drug ingestion has been shown to be exponential. The "half-life" of plasma isoniazid is a method of phenotyping, and it has been shown that this can be determined fairly accurately by two observations, the first at two hours, and the second at six hours following drug ingestion.

Another method of determining the isoniazid inactivator phenotype is by determining the six-hour plasma concentration only. When this is done by means of a chemical technique, the bimodality in the distribution of the values is rather clearer than when a microbiologic technique is used. When the same samples of blood are assayed by the chemical and microbiologic methods, then there is a strong positive correlation between the results.

When the 128 subjects studied were classified for phenotype by the chemical technique, it was found that 39 of 78 white subjects and 26 of 50 U.S. Negro subjects were rapid inactivators. This suggests that the distribution of phenotypes is similar in these two ethnic groups.

Sumario

La Determinación del Fenotipo Inactivador de la Isoniacida

Se ha observado que, en la especie humana, existen dos patrones distintos del metabolismo de la isoniacida. Algunas personas revelan después de la ingestión de la droga una rápida baja de la concentración de isoniacida en el plasma, en tanto que otras quizás no acusen más que una baja lenta. Un sujeto humano dado muestra un patrón constante. Este patrón es determinado genéticamente; el carácter mendeliano dominante de rápida inactivación pasa por ser debido a una acetilación mayor de la droga que la que existe cuando prevalece el carácter recesivo.

Se ha demostrado que la declinación en la concentración plasmática de la isoniacida a las seis horas de la ingestión de la droga es exponencial. La "hemivida" de la isoniacida plasmática representa un método para determinar el fenotipo, habiéndose demostrado que cabe hacer esto con bastante exactitud por medio de dos observaciones, la primera a las dos horas, y la segunda a las seis horas, de la ingestión de la droga.

Otro método para determinar el fenotipo inacti-

vador de la isoniacida consiste en determinar la concentración en el plasma solamente a las seis horas. Cuando se hace esto por medio de una técnica química, la bimodalidad en la distribución de los valores resulta algo más clara que cuando se usa una técnica microbiológica. Cuando se ensayan las mismas muestras de sangre con los métodos químico y microbiológico, nótase una correlación intensamente positiva en los resultados.

Cuando se clasificaron para fenotipo los 128 sujetos estudiados con la técnica química, se observó que 39 de 78 sujetos de raza blanca y 26 de 50 sujetos estadounidenses de raza negra eran inactivadores rápidos. Esto sugiere que la distribución de los fenotipos es semejante a la de estos dos grupos étnicos.

Resume

La détermination du phénotype inactivant l'isoniazide

On a trouvé qu'il y a deux sortes distinctes de métabolismes d'isoniazide présentes dans l'espèce humaine. Certaines personnes montrent une chute rapide de la concentration d'isoniazide dans le plasma après la prise du médicament, tandis que d'autres montrent seulement une chute lente. Le mode est constant pour un sujet donné. Ce mode est déterminé génétiquement; le caractère Mendèlien dominant d'inactivation rapide est, pense-t-on, dû à une acétylation du médicament plus importante que celle qui se produit en présence du caractère récessif.

La diminution de la concentration plasmatique d'isoniazide au delà de six heures après la prise du médicament est, a-t-on trouvé, une question d'interprétation. La "demi-vie" de l'isoniazide plasmatique est un procédé de détermination de phénotype, et on a montré que cela peut être déterminé passablement bien, grace à deux observations, la première deux heures après et la seconde six heures après la prise du médicament.

Une autre méthode pour déterminer le phénotype inactivant l'isoniazide est de déterminer seulement la concentration plasmatique de six heures. Quand ceci est fait au moyen d'une technique chimique, la double modalité dans la distribution des données est plutôt plus claire que celle obtenue avec l'emploi d'une technique microbiologique. Quand les mêmes échantillons de sang sont traités par des méthodes chimiques et microbiologiques, il y a alors une corrélation fortement positive entre les résultats.

Quand les 128 études de sujets furent classées par phénotype par la méthode chimique, on trouva que 39 des 78 sujets blancs et 26 des 50 sujets noirs des Etats Unis inactivaient rapidement. Ceci laisse à penser que la distribution du phénotype est semblable chez ces deux groupes ethniques.

Acknowledgments

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NUMERICAL ESTIMATES OF THE EXTENT OF THE LESION IN LUPUS VULGARIS CUTIS AND THEIR SIGNIFICANCE FOR EPIDEMIOLOGIC AND CLINICAL RESEARCH[1, 2]

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INTRODUCTION

It is of considerable importance to be able to provide a quantitative indication of the degree of severity of a disease. However, the classification often used, such as "large," "moderate," and "small," is not exact and depends very much on personal judgment. The present study proposes a much more precise method of reporting the extent of a skin disease, lupus vulgaris cutis. The significance of precise measurement is demonstrated in epidemiologic and clinical analyses. In particular, the epidemiologic analysis shows how it may be determined, on the basis of the extent of the lesion, whether a registration deficit of patients exists. In the clinical analysis, some of the factors are examined which are of significance for the extent of the lupus lesion.

MATERIAL AND METHODS

Patient material: The investigation is based on those patients admitted to the Finsen Institute, Copenhagen, during the period 1895–1954, with the diagnosis of lupus vulgaris cutis (hereinafter referred to as lupus). It is justifiable to assume that this material comprises more than 80 per cent of the cases of lupus diagnosed in Denmark throughout this period. The diagnosis was based on clinical criteria, but was frequently supported by bacteriologic and histologic evidence, the tuberculin test, and the presence of other tuberculous disease. It should be stressed particularly that the diagnosis was uniformly substantiated in cases of varying extent of lupus (for further details, *see* (1, chapter 3).

The first section of the study provides an account of the extent of the lupus lesion in the patients on admission to the Finsen Institute. A 10 per cent sample of the 3,902 registered cases of lupus was employed for this purpose, this sample comprising 390 patients, 114 male and 276 female.

In the second section of the study, some of the factors are examined which could be of significance for the extent of the lesion. These factors are related to the clinical onset of lupus. To carry out such studies, it was therefore necessary to have material comprising all cases of lupus which *started* within a definite period. This material was selected from among the patients admitted, comprising the majority of cases with the onset of lupus in the period 1895–1934, totaling 1,885 cases, 546 male and 1,339 female.[3]

Size of lesion: The present study reports the extent of the skin area affected by lupus when the patients were admitted to the Finsen Institute. If part of a lesion was cicatrized, this area is also included.

All records were examined by one of the writers (O. H.). Great emphasis was laid on avoiding bias, and its significance—for example for time trends—was strongly reduced by the order in which the case records were arranged and perused (1, chapter 2).

The evaluation of the extent of the areas was based on the medical reports in the case records and on the photographs of the lesions, which almost always accompanied the case records. In slightly less than half the number of cases, the length and breadth of the lupus lesion had been measured by the intern taking the history, and was recorded in centimeters. In such cases, the area was calculated as the product of length and breadth, as well as when the lesion was not rectangular. In the remaining patients, the entire area or a portion of it was reported with less precision, for example, as "almond-sized," or "half the cheek." A code list was worked out for such reports to ensure that uniform cases were evaluated in a uniform manner. In what follows, these areas are described as "estimated," in contrast to the areas first mentioned, which are described as "measured."

[1] From the Dermatological Department, the Finsen Institute, Copenhagen, Denmark.

[2] This study was supported by grants from Bang's Foundation, Denmark, and Weimann's Foundation, Denmark.

[3] In reference (1), chapters 8 and 13, a detailed account is given of the delimitation of patient material comprising the majority of the cases of lupus developing in the years 1895–1944. The present study comprises only cases of clinical onset in the first four decades, as the number of cases which developed during the last decade (1935–1944) was too small to permit division into the necessary groups. Among the cases arising in the years 1895–1934, a total of 86 cases were excluded, 58 because the extent of lupus was not stated and 28 because the patients were sixty-five years old or older at the onset of the lupus.

The areas were classified in the following groups: <4 cm.²; 4 cm.²–<11 cm.²; 11 cm.²–<21 cm.²; 21 cm.²–<51 cm.²; 51 cm.²–<101 cm.²; ≥101 cm.²; and a group with uncertain/unstated area.

The following investigations were made to evaluate how correctly the extent of the areas had been estimated:

(*a*) *Repeat coding:* A re-evaluation of the extent of the area was made in 208 patients (a 10 per cent sample of patients whose lupus had begun during the years 1895–1944, (1, chapters 2 and 13). As a result, agreement was found with the first grouping in all but 8 cases, which on recording were placed one group higher or lower.

(*b*) *Comparison between the extent of the measured and estimated areas:* In order to investigate whether the estimates made of the size of the lesion introduced serious errors, a comparison was made between the distributions in patients in whom the lupus area had been "measured" and "estimated," respectively. The comparison was carried out on the assumption that it was only by chance that the extent of the lesions had not been measured on admission. It is the general impression, supported among other things by the photographs, that this assumption has been satisfied. However, there has been a tendency for very extensive lesions, such as those involving a whole extremity, to be measured less frequently than other conditions.

Among the male patients, the affected area was "measured" in 47 per cent of the cases, while it was "estimated" in 53 per cent of the cases. The distribution according to size of lesion was uniform in these two groups (table 1) so that the estimates can be regarded as correct. Among the female patients, the area was "measured" in 37 per cent of the cases and "estimated" in 63 per cent; the greater frequency of "estimation" in females is because the nose was more frequently affected among female patients. The distribution curve for female patients whose size of lesion was "estimated," was displaced toward higher values than the curve for patients with "measured" areas; thus, the median was 1.5 times as great. The reason for this is that, when "estimating," the same standards are used for men and women, although women are on the average smaller than men. This systematic error, however, is independent of the residence of the patient, the time of onset of the lupus, et cetera, and can therefore not explain the differences between the various groups, which are described below.

Results

Size of the Lesion on Admission

Among the 390 patients who are a representative sample of the patients admitted during the

TABLE 1

Lupus Patients* in Whom the Extent of Lesion Was "Measured"/"Estimated,"† Distributed According to the Extent of Lesion on Admission to the Finsen Institute

Area (cm.²)	Males			Females			Total		
	Measured Area	Estimated Area	Total	Measured Area	Estimated Area	Total	Measured Area	Estimated Area	Total
	Number of Patients								
0–3	69	62	131	202	212	414	271	274	545
4–10	49	76	125	129	230	359	178	306	484
11–20	43	46	89	71	166	237	114	212	326
21–50	59	53	112	73	158	231	132	211	343
51–100	31	28	59	32	48	80	63	76	139
101+	27	51	78	29	86	115	56	137	193
Total	278	316	594	536	900	1,436	814	1,216	2,030
	Cumulative Function in Percentage								
<4	25	20	22	38	24	29	33	23	27
<11	42	44	43	62	49	54	55	48	51
<21	58	58	58	75	68	70	69	65	67
<51	79	75	77	89	85	86	85	82	84
<101	90	84	87	95	90	92	93	89	90

* Lupus patients in whom lupus developed in the period 1895–1944, exclusive of 62 in whom the area was not stated/uncertain.

† See text page 862.

TABLE 2

LUPUS PATIENTS* DISTRIBUTED ACCORDING TO THE EXTENT OF LESION ON ADMISSION TO THE FINSEN INSTITUTE AND ACCORDING TO AGE ON ADMISSION

Area (cm.²)	Age on Admission (*years*)								
	Males			Females			Total		
	0–24	25+	Total	0–24	25+	Total	0–24	25+	Total
Number of Patients									
0–3	16	1	17	31	29	60	47	30	77
4–10	11	8	19	27	34	61	38	42	80
11–20	6	4	10	14	28	42	20	32	52
21–50	16	12	28	8	20	28	24	32	56
51–100	4	8	12	6	18	24	10	26	36
101+	8	16	24	10	32	42	18	48	66
Uncertain/unstated	2	2	4	9	10	19	11	12	23
Total	63	51	114	105	171	276	168	222	390
Cumulative Function in Percentage									
<4	26	2	15	32	18	23	30	14	21
<11	44	18	33	60	39	47	54	34	43
<21	54	27	42	75	57	63	67	50	57
<51	80	51	67	83	69	74	82	65	72
<101	87	67	78	90	80	84	89	77	82

* Ten per cent sample of all lupus patients admitted to the Finsen Institute during the period 1895–1954.

years 1895–1954, the size of the lesion varied between 0.3 cm.² and about 4,000 cm.² Using the arithmetic mean to characterize the extent of the lupus, the very extensive cases will result in a high value. Examining the *distributions*, however, it is striking that a large number of the lesions are in fact of small extent. Preference was given, therefore, to working with the distribution of the size of the lesion in the various patient groups, as thereby a much more relevant picture is obtained.

In 21 per cent of the patients, the size of the lesion was less than 4 cm.² on admission, and in 18 per cent it was 101 cm.² or more; the median of the distribution was 15 cm.² On the whole, the lesion was more extensive among the male patients than among the female patients; thus, the median was twice as great (table 2, figure 1).

Lupus showed on the whole more extensive lesions among the patients who were twenty-five years of age or older on admission than among the younger patients; thus, the median was two to three times greater (table 2, figure 2). The reason for this is that there are relatively more protracted cases among the older patients and that, *ceteris paribus*, lupus shows on the whole a more extensive lesion in adults than in children (*see* below).

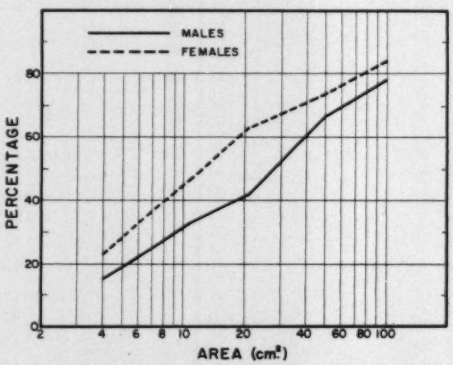

FIG. 1. Lupus patients distributed according to the extent of lesion on admission to the Finsen Institute. (Data are given in table 2.)

In general, the lupus lesion was larger among patients from the provinces than among patients

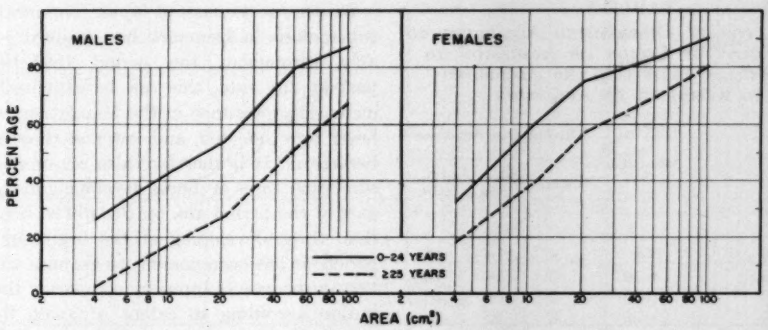

FIG. 2. Lupus patients distributed according to the extent of lesion on admission to the Finsen Institute and according to age on admission. (Data are given in table 2.)

from the capital (table 3, figure 3). To some degree, this is because there are relatively more older persons among patients from the provinces. This cannot explain the whole difference, however, as the same result is found in each of the age groups examined.[4] If the patients from the provinces had shown relatively more cases of prolonged disease, this would have been a simple explanation of the observed difference, but this was not found (1, chapter 7). It has therefore been reasonable to assume that there is a deficit of provincial patients with small lesions (*see* Discussion).

Factors of Significance for the Extent of the Lesion on Admission

The temporal interval between the onset of lupus and the admission of the patient to the Finsen Institute: The longer the time that has elapsed between the onset of the lupus and the admission of the patient to the Finsen Institute, the greater has been the area of the lupus lesion on the average (table 4, figure 4). Thus, among the patients for whom the interval had been twenty-one years or more, the median was four to five times as great as among those for whom the interval was less than two years. This tendency was also found when the patients were grouped according to age at the onset of lupus and according to the temporal relation of the lupus to the first tuberculous disease.

[4] In view of the size of the material, the analysis was made for female patients only; these were divided into two age groups, those less than twenty-five years and those twenty-five years and older.

In the groups compared, the relative composition has varied with regard to the interval noted; thus, there were relatively more long intervals among those who had been children when the lupus started than among those who had been older; and this fact alone could result in a considerable difference between the groups. It has therefore been necessary to carry out a standardization, so that the relative composition with respect to cases of varying interval is the same in all groups.[5]

Age of patient at the onset of lupus: The disease has generally been less extensive in those patients who were children when the lupus developed than in those who were adults (table 5). This can be explained since most of them were admitted to the Finsen Institute while they were still children. There has been a tendency to less extensive disease among those who were fifteen to thirty-four years of age at the onset of the disease than among those who were thirty-five to sixty-four years of age when the lupus appeared; this was found to be the case among male patients for all groups examined, but among female patients only when lupus was the first tuberculous disease.

Temporal relation between lupus and the first tuberculous disease: On the whole, the disease was of lesser extent in those patients in whom lupus was the first tuberculous disease than among those who developed lupus simultaneously with or after another tuberculous disease

[5] The following distribution has been used: 0–<2 years, 35.3 per cent; 2–<6 years, 31.5 per cent; 6–<21 years, 26.7 per cent; and >21 years, 6.4 per cent.

TABLE 3

LUPUS PATIENTS* DISTRIBUTED ACCORDING TO THE EXTENT OF LESION ON ADMISSION TO THE FINSEN INSTITUTE AND ACCORDING TO RESIDENCE ON ADMISSION

	Area (cm.²)	Residence on Admission		
		Capital	Provinces	All Denmark
Males				
Number of patients	0–3	8	9	17
	4–10	3	16	19
	11–20	2	8	10
	21–50	6	22	28
	51–100	2	10	12
	101+	6	18	24
	Uncertain/ unstated	—	4	4
	Total	27	87	114
Cumulative function in percentage	<4	30	11	15
	<11	41	30	33
	<21	48	40	42
	<51	70	66	67
	<101	78	78	78
Females				
Number of patients	0–3	20	40	60
	4–10	23	38	61
	11–20	6	36	42
	21–50	3	25	28
	51–100	2	22	24
	101+	6	36	42
	Uncertain/ unstated	5	14	19
	Total	65	211	276
Cumulative function in percentage	<4	33	20	23
	<11	72	40	47
	<21	82	58	63
	<51	87	71	74
	<101	90	82	84

* Ten per cent sample of all lupus patients admitted to the Finsen Institute during the period 1895–1954.

(table 5). Such a difference might be explained by the fact that resistance in the first-mentioned group was higher than in the two latter groups. However, an investigation of the fatality ratio in these patient groups does not suggest that there was any essential difference (1, chapter 10). The difference might also be attributed to differences in the period of the onset of lupus, but this has not been observed.[6]

[6] The patients were divided into two groups: those with the onset of lupus in 1895–1914 and those with the onset of lupus in 1915–1934. The analysis, which was made according to sex and age, showed a uniform distribution for the various groups.

Decade for the onset of lupus: The incidence of tuberculosis in Denmark has declined considerably throughout the period 1895–1934. In part at any rate, this can be explained by an increasing resistance in the population, whereby fewer were infected, and fewer of those infected became ill. It is therefore also reasonable to assume that cases of illness developing in the latter part of the period are, on the whole, less serious than those developing at the beginning of the period. It has been possible to examine this question in the case of lupus by comparing the distribution according to extent of lesion for cases developing in the decade 1895–1904 with the distribution for cases developing in the subsequent decades. The investigation is limited to patients admitted to the Institute less than six years after the onset of lupus, as on the average these patients have been admitted in the same decade in which the lupus developed. In table 6 and figure 5 it may be seen that there has been a tendency for the disease to become of lesser extent in the course of time; thus, during the last decade the median was only half as great as during the first decade. In reality, the change has been even greater: In the course of time, an increasing proportion of the cases that developed have belonged to older age groups; lupus in these groups is, on the average, of greater extent than lupus in the younger age groups, so that, other things being equal, the age displacement has exerted a tendency in the direction opposite to that of the observed time trend.[7]

DISCUSSION

The method of measuring and grouping lesions of different extent which is employed in the present paper is considered far more suitable than those generally used, being more exact and independent of personal estimate. This also

[7] Thus, in the first decade, 53 per cent of the male and 36 per cent of the female patients were less than fifteen years of age at the onset of lupus, and 10 and 20 per cent, respectively, were between the ages of thirty-five and sixty-four years. During the last decade, the percentages of male and female children were 32 and 19 per cent, respectively, while the percentages of older persons were 25 and 45 per cent, respectively.

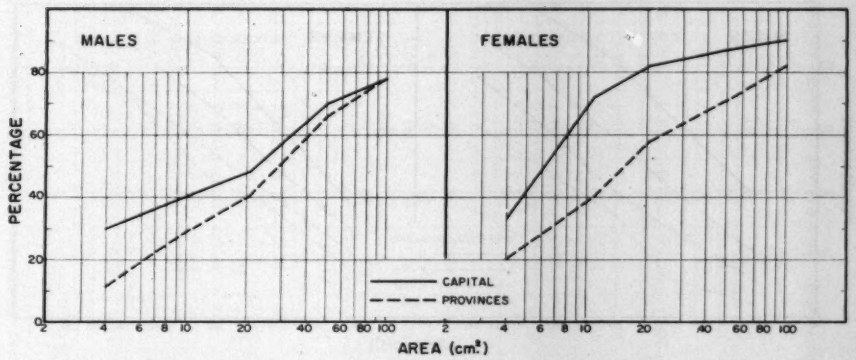

FIG. 3. Lupus patients distributed according to the extent of lesion on admission to the Finsen Institute and according to residence on admission. (Data are given in table 3.)

TABLE 4

LUPUS PATIENTS* DISTRIBUTED ACCORDING TO THE EXTENT OF LESION ON ADMISSION TO THE FINSEN INSTITUTE AND ACCORDING TO THE INTERVAL FROM ONSET OF LUPUS TO ADMISSION

Area (cm.²)	Interval (*years*)											
	Males				Females				Total			
	<2	2–5	6–20	>21	<2	2–5	6–20	>21	<2	2–5	6–20	>21
	Number of Patients											
0–3	64	34	17	2	192	99	63	10	256	133	80	12
4–10	45	34	28	4	168	85	64	16	213	119	92	20
11–20	30	27	18	10	104	63	44	6	134	90	62	16
21–50	27	35	37	8	74	72	56	18	101	107	93	26
51–100	2	20	22	6	8	17	42	5	10	37	64	11
101+	1	17	37	19	7	34	51	17	8	51	88	36
Total.....	169	167	159	49	553	370	320	72	722	537	479	121
	Cumulative Function in Percentage											
<4	38	20	11	4	35	27	20	14	35	25	17	10
<11	64	41	28	12	65	50	40	36	65	47	36	26
<21	82	57	40	33	84	67	53	44	84	64	49	40
<51	98	78	63	49	97	86	71	69	98	84	68	61
<101	99	90	77	61	99	91	84	76	99	91	82	70

* Patients in whom lupus developed in the period 1895–1934.

holds true in comparisons between statistics from different countries, as the measuring unit (the square centimeter) is international and exact. Evaluation of the extent of the lesions has been hindered by lack of material, indicating, for example, the size of a normal cheek or a normal ear. If such data could be prepared, it would increase the precision of the method.

In therapeutic investigations, it is important to be able to measure the extent of the disease. For example, if the effectiveness of various forms of treatment has to be compared, it is necessary to ensure that the cases in the various groups are distributed uniformly according to degree of severity. In many forms of treatment, it is also of interest to show how far it has been possible to reduce the extent of the disease.

In epidemiologic studies, analyses of the de-

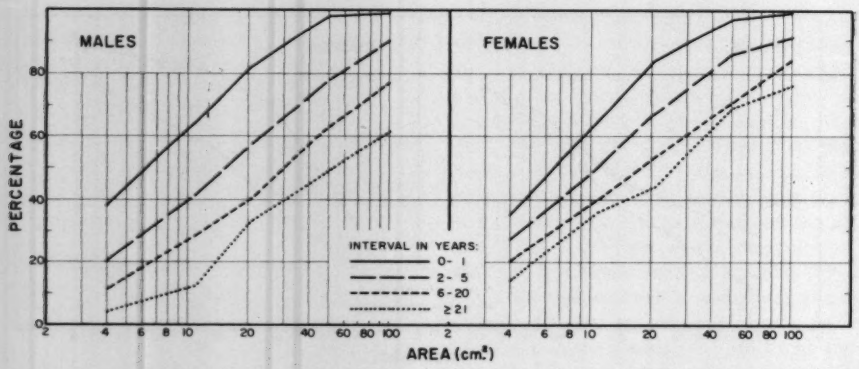

FIG. 4. Lupus patients distributed according to the extent of lesion on admission to the Finsen Institute and according to the interval from onset of lupus to admission. (Data are given in table 4.)

gree of severity of cases can be used to investigate whether an omission to notify patients has occurred. It was found in the present study that lupus cases from the provinces were generally more severe than lupus cases from the capital. This may be because a number of minor cases from the provinces were not sent to the Finsen Institute for treatment, but an attempt was made to treat them locally. The magnitude of such a deficit has been evaluated as follows: If the number of lupus patients from the provinces with lesions of smaller size than 11 cm.² is increased by 160, the distribution according to size becomes the same as that of the patients from the capital. This implies that there is a deficit of patients from the provinces amounting to $\frac{160}{160 + 296}$ = 34 per cent.

Correspondingly, the tendency to relatively more extensive cases among men can be considered as the result of men seeking treatment for smaller lesions of the skin less frequently than women. If the distribution according to size is in fact uniform in the two sexes, it can be calculated that there is a 15 per cent deficit of male patients. It should be stressed that, even if such a deficit were present, it would not be enough to explain the great relative difference in incidence which has been observed in the two sexes (1, chapters 4 and 6).

It was striking that the lesion was of small extent in a large proportion of the patients. From a cosmetic point of view, therefore, the disease may be regarded as relatively benign, even when protracted, possibly involving tissue destruction and often localized to head or neck. The greatest significance of lupus lies in the fact that it is a symptom of a tuberculous infection which often runs a serious course (1, chapters 10, 11).

Generally speaking, the interval between the onset of the lupus and the admission of a patient to the Finsen Institute can be considered as correctly reported (1, chapter 7). It has therefore been striking that the lupus lesion could be both very wide in extent after a brief course and still of small extent after a disease period of many years. In pulmonary tuberculosis, these two types correspond to "galloping consumption" and "benign chronic tuberculosis."

SUMMARY

The extent of the lesion of lupus vulgaris cutis has been studied in patients with the disease who have been admitted to the Finsen Institute, Denmark. A detailed account is given of the method of measurement, including control of accuracy.

A 10 per cent sample was taken of the patients admitted to the Institute during the period 1895–1954. The sample comprised 390 patients, 114 males and 276 females. In 21 per cent of the patients, the lesion was less than 4 cm.² on admission; and in 18 per cent it was 101 cm.² or more; the median of the distribution was 15 cm.² Generally, lupus was of greater extent in male patients than in female patients, and in patients from the provinces than in patients from the capital.

Factors of significance for the extent of the

TABLE 5

LUPUS PATIENTS* DISTRIBUTED ACCORDING TO THE EXTENT OF LESION ON ADMISSION TO THE FINSEN INSTITUTE AND ACCORDING TO AGE AT THE ONSET OF LUPUS. THE DISTRIBUTIONS ARE GIVEN ACCORDING TO THE TEMPORAL RELATION BETWEEN LUPUS AND OTHER TUBERCULOUS DISEASES IN THE PATIENTS

	Area (cm.²)	Lupus First Tuberculous Disease†				Lupus Developed Simultaneously with Another Tuberculous Disease				Lupus Developed after Another Tuberculous Disease				Total			
Age on Onset of Lupus (*years*)		0–14	15–34	35–64	Total	0–14	15–34	35–64	Total	0–14	15–34	35–64	Total	0–14	15–34	35–64	Total
Males																	
Number of patients	0–3	52.0	17.9	13.8	83.7	13.3	7.9	2.1	23.3	9.7	8.7	7.1	25.5	75.0	34.5	23.0	132.5
	4–10	29.7	11.4	2.6	43.7	8.0	11.3	2.4	21.7	14.5	23.3	4.6	42.4	52.2	46.0	9.6	107.8
	11–20	15.0	9.3	6.6	30.9	3.1	5.1	1.7	9.9	6.6	24.3	6.0	36.9	24.7	38.7	14.3	77.7
	21–50	16.8	11.7	2.3	30.8	10.5	9.0	4.4	23.9	13.1	28.5	10.1	51.7	40.4	49.2	16.8	106.4
	51–100	8.7	3.4	8.2	20.3	6.4	2.3	0.1	8.8	5.6	14.1	4.5	24.2	20.7	19.8	12.8	53.3
	101+	13.8	4.2	2.7	20.7	5.8	2.4	4.2	12.4	4.6	17.1	9.9	31.6	24.2	23.7	16.8	64.7
	Total	136.0	57.9	36.2	230.1	47.1	38.0	14.9	100.0	54.1	116.0	42.2	212.3	237.2	211.9	93.3	542.4
Cumulative function in percentage	<4	38	31	38	36	28	21	14	23	18	8	17	12	32	16	25	24
	<11	60	51	45	55	45	51	30	45	45	28	28	32	54	38	35	44
	<21	71	67	64	69	52	64	42	55	57	49	42	49	64	56	50	59
	<51	83	87	70	82	74	88	71	79	81	73	66	74	81	79	68	78
	<101	90	93	93	91	88	94	72	88	91	85	77	85	90	89	82	88
Females																	
Number of patients	0–3	124.6	61.4	29.9	215.9	31.4	14.3	13.9	59.6	15.5	33.4	31.8	80.7	171.5	109.1	75.6	356.2
	4–10	49.8	46.8	15.3	111.9	17.1	27.6	16.2	60.9	20.0	68.0	57.5	145.5	86.9	142.4	89.0	318.3
	11–20	22.1	11.7	28.7	62.5	10.3	13.7	8.2	32.2	13.5	63.7	46.6	123.8	45.9	89.1	83.5	218.5
	21–50	12.2	21.1	12.9	46.2	7.8	31.6	14.4	53.8	18.3	58.8	45.9	123.0	38.3	111.5	73.2	223.0
	51–100	6.5	4.7	3.0	14.2	6.0	3.8	9.7	19.5	9.8	22.8	19.1	51.7	22.3	31.3	31.8	85.4
	101+	11.3	12.3	13.9	37.5	7.4	1.9	3.6	12.9	8.4	27.5	27.2	63.1	27.1	41.7	44.7	113.5
	Total	226.5	158.0	103.7	488.2	80.0	92.9	66.0	238.9	85.5	274.2	228.1	587.8	392.0	525.1	397.8	1,314.9
Cumulative function in percentage	<4	55	39	29	44	39	15	21	25	18	12	14	14	44	21	19	27
	<11	77	68	44	67	61	45	46	50	42	37	39	38	66	48	41	51
	<21	87	76	71	80	74	60	58	64	57	60	60	60	78	65	62	68
	<51	92	89	84	89	83	94	80	86	79	82	80	80	87	86	81	85
	<101	95	92	87	92	91	98	95	95	90	90	88	89	93	92	89	91

* Patients in whom lupus developed in the period 1895–1934.
A standardization has been carried out, so that the distribution according to the interval from onset of lupus to admission is the same in all groups.
† Including patients in whom lupus was the only form of tuberculosis.

TABLE 6

LUPUS PATIENTS* DISTRIBUTED ACCORDING TO THE EXTENT OF LESION ON ADMISSION TO THE FINSEN INSTITUTE AND ACCORDING TO THE DECADE FOR ONSET OF THE LUPUS

	Area (cm.²)	Decade for Onset of Lupus			
		1895–1904	1905–1914	1915–1924	1925–1934
Males					
Number of patients	0–3	22.3	30.3	28.5	34.7
	4–10	19.4	23.8	23.3	11.6
	11–20	16.5	14.4	16.2	11.0
	21–50	20.1	11.1	15.8	14.2
	51–100	11.8	7.1	3.9	6.8
	101+	5.3	8.5	3.0	2.1
	Total	95.4	95.2	90.7	80.4
Cumulative function in percentage	<4	23	32	31	43
	<11	44	57	57	58
	<21	61	72	75	71
	<51	82	84	92	89
	<101	94	91	97	97
Females					
Number of patients	0–3	54.9	71.8	94.9	67.4
	4–10	54.6	70.4	63.7	43.2
	11–20	46.2	48.6	39.1	20.3
	21–50	43.6	42.4	38.1	12.8
	51–100	8.1	10.5	3.3	2.8
	101+	13.8	10.6	9.3	8.4
	Total	221.2	254.3	248.4	154.9
Cumulative function in percentage	<4	25	28	38	44
	<11	50	56	64	71
	<21	70	75	80	85
	<51	90	92	95	93
	<101	94	96	96	95

* Patients in whom lupus had its onset in the period 1895–1934 and who were admitted to the Finsen Institute less than six years after the onset of the lupus.

A standardization has been carried out, so that the ratio between the number of patients in whom the interval was less than two years and the number of patients in whom the interval was two to five years is the same in all groups.

lupus lesion have been studied in 1,885 cases, 546 males and 1,339 females. This material comprises a majority of cases in which lupus had its onset during the period 1895–1934, and is selected from among the patients admitted. The longer the time between the onset of the lupus and the admission of the patient to the Finsen Institute, the greater has been the extent of the lupus in general. The older the patient at the onset of the lupus, the more extensive the disease has been. If the lupus occurred after or simultaneously with other tuberculous disease, the disease was generally of greater extent than if the lupus was the first tuberculous disease. Lupus which had its onset during the first decades of the period was generally of greater extent than lupus which developed during the later decades.

On the basis of the distributions described here, it is shown how to investigate whether a deficit of cases exists, and how to evaluate the magnitude of this deficit.

SUMARIO

Cómputos Numéricos del Tamaño de la Lesión en el Lupus Vulgar del Cutis y Su Importancia para la Investigación Epidemiológica y Clínica

Se ha estudiado la magnitud de la lesión del lupus vulgar del cutis en enfermos recibidos en el

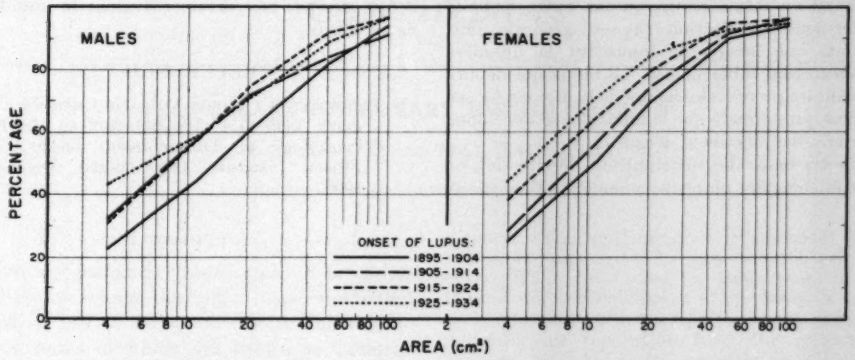

FIG. 5. Lupus patients distributed according to the extent of lesion on admission to the Finsen Institute and according to the decade for onset of the lupus. (Data are given in table 6.)

Instituto Finsen, de Dinamarca. Se ofrece una reseña pormenorizada del método de medición, que comprende una fiscalización de la exactitud.

Se tomó una muestra de 10 por ciento de los enfermos ingresados durante el período de 1895–1934. La muestra comprendió 390 enfermos, 114 varones y 276 mujeres. En 21 por ciento de los sujetos, la lesión tenía menos de 4 cm.² al ingreso, y en 18 por ciento medía 101 cm.² o más; el promedio de la distribución era 15 cm.². En general, se encontró lupus en mayor proporción en el sexo masculino que en el femenino, y en los enfermos procedentes de las provincias más que en los de la capital.

Se estudiaron factores de importancia en cuanto al tamaño de la lesión luposa en 1,885 casos: 546 en varones y 1,339 en mujeres. Este material comprende una mayoría de casos en los que el lupus tuvo su iniciación durante el período de 1895–1934, y ha sido seleccionado de entre los enfermos recibidos. Mientras más largo ha sido el tiempo transcurrido entre la iniciación del lupus y el ingreso del enfermos en el Instituto Finsen, mayor ha sido la extensión del lupus en general. Mientras más viejo era el enfermo al iniciarse el lupus, más extensa se mostró la enfermedad. Si el lupus se presentaba después de otra afección tuberculosa o simultáneamente con ésta, más difusa era generalmente que si era la primera afección tuberculosa. El lupus que tuvo su iniciación durante los primeros decenios del período alcanzó generalmente mayor tamaño que el que se presentó durante los últimos decenios.

A base de la distribución descrita, se muestra cómo se puede averiguar si existe un déficit de casos y cómo justipreciar la magnitud de dicho déficit.

RESUME

Estimations numériques de l'extension de la lésion dans le lupus vulgaris cutis, et leur signification dans les recherches épidémiologiques et cliniques

L'extension de la lésion de lupus vulgaris cutis a été étudiée chez des sujets ayant cette maladie et qui furent admis à l'Institut Finsen, Danemarque. Un accompte détaillé est fourni sur cette méthode de mesure, y compris le contrôle de sa fidèlité.

Un échantillon de 10% fut pris chez des malades admis pendant la période 1895–1954. L'échantillon comprit 390 malades, 114 hommes et 276 femmes. Chez 21% des malades, la lésion fut moins de 4 cm.² de dimension à l'admission; et chez 18%, il y eut 101 cm.² ou plus; la moyenne de distribution fut de 15 cm.². On trouva, d'une façon générale, le lupus plus étendu chez les malades hommes que chez les malades femmes, et chez les malades de province plutôt que chez ceux de la capitale.

Les facteurs d'importance en ce qui concerne l'extension de la lésion de lupus ont été étudiés chez 1.885 cas, 546 hommes et 1.339 femmes. Ce matériel comprit une majorité de cas dans lesquels le lupus débuta pendant la période 1895–1934, et fut sélectionné parmi les malades admis. Plus la période comprise entre le début du lupus et l'admission du malade à l'Institut Finsen, a été longue, plus le lupus a été étendu d'une façon générale. Plus le malade était agé au début du lupus, plus la maladie a été extensive. Quand le lupus survint

après ou en même temps qu'une autre maladie tuberculeuse, l'affection fut en générale plus étendue que lorsque le lupus fut la première manifestation tuberculeuse. Le lupus qui débuta pendant les premières décades de la période fut, en général, plus étendu que le lupus qui se développa au cours des dernières décades.

Sur les bases des distributions décrites ici, on montre comment on peut rechercher s'il existe un déficit des cas et, comment évaluer la grandeur de ce déficit.

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Case Reports

PRIMARY PULMONARY HISTIOCYTOSIS X[1]

L. S. ARANY[2] AND MAX BAUMWELL

(Received for publication May 13, 1960)

INTRODUCTION

Primary pulmonary histiocytosis X or eosinophilic granuloma of the lung is considered to be a localized form of a systemic disease, the disseminated forms of which are known as Letterer-Siwe's disease and Hand-Schueller-Christian syndrome. It usually involves bones and viscera but, occasionally, may be confined to one system only. Involvement of the lungs alone appears to be rare. Nadeau and associates (1) recently reported seven cases which were seen at the Mayo Clinic between July, 1953, and July, 1959. These writers collected 21 more cases from the literature and summarized the data, listing age, sex, complications, time of onset to diagnosis, follow-up period after diagnosis, and the clinical status of the patients at the end of the follow-up study. By personal contact with a number of chest physicians and by attendance at various meetings, where such cases were presented, it is the impression of the present writers that there is a fair number of unpublished cases, and this entity is probably more common than would appear from the number of the patients whose cases were reported in the literature. In view of this, there would be little justification for reporting the present case were it not for the availability of a long-term follow-up study which is believed to be essential for the appraisal of the natural history of this condition. At the time when this case was diagnosed, there were only four known cases of pulmonary histiocytosis.

CASE REPORT

A 24-year-old white male hard-rock miner was admitted to the Veterans Hospital, Walla Walla, Washington, on September 13, 1954, with complaints of minimal productive cough and mild pain in the right lower chest which did not increase on deep inspiration. He also had slight exertional dyspnea. The patient first experienced these symptoms approximately three months prior to entry. While he worked for three years in the mines before his enlistment in the Army in April, 1951, and continued working for about one year following his separation from the service in January, 1953, his actual exposure to silica dust was very limited. In February, 1954, a routine chest film was made and the patient was told that he had silicosis and he was not allowed to work under ground.

Physical examination revealed a well-developed and well-nourished young man who did not appear ill. The temperature was 98°F.; pulse 76 per minute; blood pressure, 120 systolic, 82 diastolic in mm. of mercury. A chest examination revealed normal configuration, symmetrical respiratory expansions, and normal resonance. The breath sounds were slightly harsh; no rales or rhonchi were noted. There was no lymphatic adenopathy or bone tenderness, and there were no other physical abnormalities. A roentgenogram of the chest disclosed fine nodular, partially confluent densities throughout both lungs, with multiple small cystic areas symmetrically scattered throughout both lung fields. Roentgenograms of the skeletal system as well as a gastrointestinal series in January, 1955, and November, 1956, revealed no disease. The hemoglobin was 16.5 gm.; the erythrocyte count was 4,950,000 per cu. mm.; the leukocyte count, 14,400 per cu. mm., with 75 per cent neutrophils and 1 per cent eosinophils. The sedimentation rate was 3 mm. in one hour (Cutler). The total protein was 8.2 gm. per 100 ml.; the albumin, 6.0 gm. per 100 ml.; and a urinalysis was normal. Sputum was negative on concentrate and culture for *M. tuberculosis* and pathogenic fungi. Gastric cultures were also negative. No tumor cells were found in the sputum. The reaction to intermediate-strength PPD was negative; to second-strength PPD, the reaction was weakly positive. Histoplasmin and coccidioidin skin tests were negative. A Kveim test was negative, even after six months. The bone marrow was cellular with no significant qualitative changes. Lupus erythematosus cells were not found in the bone marrow or in the blood. The Thorn test showed no depression of the number of circulating eosinophils following injection of corticotropin. The vital capacity was 3,200 cc. Bronchoscopy revealed no abnormality, and there were no malignant cells in the bronchial secretions. Scalene node biopsies were done on both sides and

[1] From the Pulmonary Disease Service, Veterans Administration Hospital, Walla Walla, Washington.

[2] Present address: Veterans Hospital, Excelsior Springs, Missouri.

disclosed reticulum cell hyperplasia. The clinical course was afebrile and uneventful. The mild cough and the right chest pain subsided soon; minimal dyspnea on exertion persisted.

On January 6, 1955, a biopsy of the right lung was performed. The entire right lung showed scattered small areas of fibrosis. The underlying lung parenchyma contained multiple, small, somewhat firm nodules measuring between 3 and 5 mm. in diameter. The nodules appeared to be grayish in color. There was no gross evidence of caseation. There were no adhesions and no increase in pleural fluid. A wedge resection of the inferior portion of the right middle lobe was performed, with removal of a piece of lung tissue measuring approximately 5 by 2 by 0.5 cm.

The specimen, after review by two pathologists who did not make a definite diagnosis, was forwarded to the Armed Forces Institute of Pathology. The following report was received:

> The histologic sections (figure 1) consist of small pieces of lung tissue containing several small, poorly circumscribed focal lesions within which the pulmonary architecture is largely or completely obliterated. These lesions consist predominantly of large, pale cells of the histiocyte type showing occasional mitotic figures. Among these cells are scattered a few to many eosinophils together with a variable number of lymphocytes and plasma cells. A few lipid-laden foamy mononuclear cells and an occasional multinucleated giant cell are also present in some of the lesions. One or two asteroids within giant cells are seen at the periphery of the lesions. One of the larger lesions is undergoing fibrosis in small foci. In the neighborhood of these lesions there is a variable degree of thickening of alveolar septa by infiltration with lymphocytes, plasma cells, and occasional eosinophils. Some of these septa show fibrous thickening and some are lined by hypertrophied alveolar cells. Alveolar macrophages are present in some alveolar lumens as well as a few lymphocytes and an occasional eosinophil. Between the lesions there is a small amount of relatively normal lung tissue. The pleura overlying some of the lesions shows slight fibrous thickening

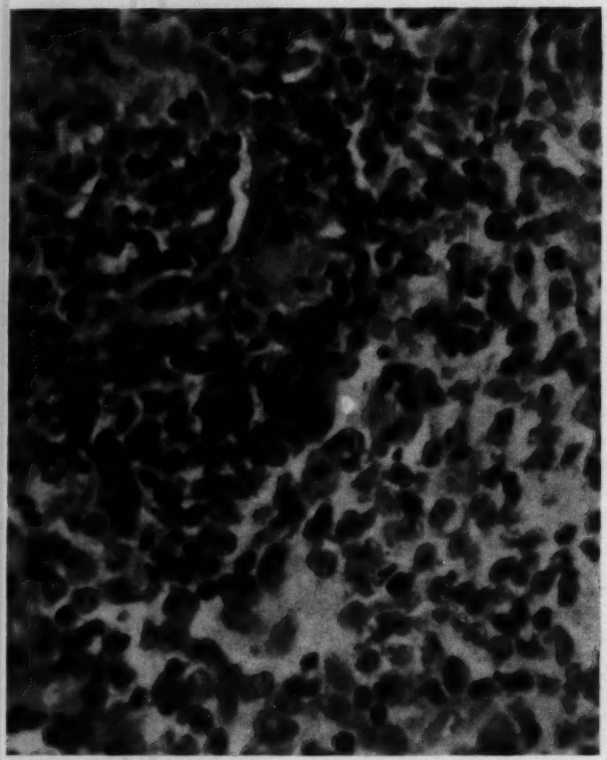

FIG. 1. Biopsy specimen, right lung, showing histiocytes, lymphocytes, plasma cells, and eosinophils. (Hematoxylin-eosin stain, 450 X)

and infiltration with small, round cells, and a pleural tag is present at one point.

The postoperative course was uneventful. The patient's condition remained excellent and he was discharged on May 20, 1955, as having received maximal hospital benefit. He received no therapy and his progress was followed by the Veterans Administration Hospital, Walla Walla, Washington, private physicians, and by a Veterans Administration regional office. His general condition remained excellent; he completed a four-year course at a university and is now gainfully employed by an industrial company.

A review of chest films made in the military service revealed that the army induction film made on October 4, 1950, appeared to be entirely normal, whereas the army separation film dated January 21, 1953, showed nodular changes most prominent in the left upper lung field and in both parahilar regions. The diffuse nodular process was considerably more prominent on the hospital admission film of September 13, 1954, and had shown slight gradual regression on subsequent serial films.

Comments

A seven-year follow-up study in a case of pulmonary histiocytosis X revealed an entirely benign course, although neither X-ray therapy nor cortisone nor cortisone-like compounds were used. So far none of the three major complications (cor pulmonale, recurring episodes of spontaneous pneumothorax, or diabetes insipidus) have occurred in this case. The history of hard-rock mining is of particular interest, as it led to the erroneous diagnosis of silicosis. This reveals the importance of a detailed inquiry into the patient's occupational history before a diagnosis of silicosis is established in cases in which a chest roentgenogram discloses a diffuse nodular lesion. Although the patient in this case worked in a hard-rock mine, he was not assigned to underground work for an appreciable length of time. The disease process remained localized to the lungs. No cases of subsequent dissemination to other systems have been reported so far in the literature.

Summary

A histologically proved case of primary pulmonary histiocytosis X is presented with data from a seven-year follow-up study which began after the first evidence of roentgenographic changes, and about five and one-half years after the development of the first symptoms. The patient's chest roentgenogram was normal twenty-six months prior to the first film revealing abnormalities. The patient received no treatment, and the course has been benign.

Sumario

Histiocitosis X Pulmonar Primaria

Se presenta un caso comprobado histológicamente de histiocitosis X pulmonar primaria con datos derivados de un período de siete años de observación, que comenzó después del primer signo de alteraciones radiográficas y unos cinco años y medio después de la aparición de los primeros síntomas. La radiografí torácica del enfermo veintiséis meses antes de la primera película patológica fué normal. El enfermo no recibió tratamiento y la evolución ha sido benigna.

Resume

Histiocytose X primaire du poumon

Un cas d'histiocytose X primaire du poumon, prouvée histologiquement, est présenté avec les renseignements apportés par une étude de contrôle de sept ans, qui commença après la première évidence de changements radiologiques, et à peu près cinq ans et demi après le développement des premiers symptômes. La radiographie pulmonaire du malade vingt six mois avant la première radiographie pathologique fut normale. Le malade ne reçut aucun traitement, et l'évolution a été bénigne.

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NEUROGENIC MUSCULAR APLASIA (EVENTRATION) OF THE DIAPHRAGM[1]

SOMCHAI BOVORNKITTI, PRASERT KANGSADAL, SOOD SANGVICHIEN, AND KASARN CHATIKAVANIJ

(Received for publication July 11, 1960)

Introduction

This is a complete report of a case of neurogenic muscular aplasia of the diaphragm, diagnosed clinically during life. The diagnosis was subsequently confirmed at operation for repair of the lesion and ultimately by histologic study of biopsy specimens. The biopsy findings support the writers' suggestions on certain aspects of etiology and pathology and on the proposal for a new name. A short account of an extensive review (1) of the condition is given, followed by a case report.

Observations

Nomenclature

Although Jean Louis Petit (2) was credited with first describing the condition found at autopsy, it was Béclard (3) who gave the name "eventration" to the disease almost a hundred years later. Since then a large number of synonyms have appeared in the literature. In the writers' previous communication (1), a new term "neurogenic muscular aplasia of the diaphragm" was proposed. This term is defined as a congenital developmental defect of the diaphragmatic musculature secondary to a quantitative defect in both spinal cord cells and nerve fibers.

Pathology

The abnormality may involve a portion or the entire leaf of one or both diaphragms. The line of attachment of the affected diaphragm has always been about the normal levels. The pleura and peritoneum have never been found to be abnormal. The fibromuscular plate varies from a layer of connective tissue containing some muscle, seen either by the naked eye or in histologic sections, to a thin translucent aponeurotic membrane. The medial and central portions of the leaf are more frequently affected, leaving the periphery uninvolved. Occasionally, involvement of only the peripheral portion has been observed. The muscular tissue, while sparse in amount, is morphologically normal and not atrophic, although it may show evidence of underdevelopment.

The phrenic nerve is small, but the individual fibers are normal. There is no evidence of degeneration in the nerve fibers or in their nuclei of origin. Total counts of nerve fibers and cells of the central cell group in the cervical cord on the affected side at appropriate levels give a much smaller number than those on the other side and in the control.

Several associated congenital anomalies have been reported from both intra- and extrathoracic organs. The lung on the affected side is usually hypoplastic as well as abnormal in other respects.

Diagnosis

In uncomplicated cases, the majority of patients are either completely asymptomatic or have long symptom-free periods. The usual symptoms are gastrointestinal and, less frequently, cardiorespiratory. The latter occur more often in the newborn and are the cause of death.

Careful physical examination of the chest should furnish clues to the diagnosis. The "Hoover's sign" (4), exaggeration of the inspiratory widening of the subcostal angle and the outward movements of the costal margin on one side, indicates weakness of the diaphragmatic action on that side. Other findings are complementary.

Conventional postero-anterior chest roentgenograms usually suggest suspicion of the anomaly. Lateral or oblique views are useful for confirming the visualization of the smooth contour of the elevated diaphragm. Barium study in various postures differentiates the anomaly from diaphragmatic hernia. The introduction of a small amount of air into the peritoneal cavity is another method used to distinguish between elevation of the diaphragm and the true hernia. In addition, the use of artificial pneumoperitoneum is of practical value in order to obtain the roentgenographic visualization of the thinness of the diseased diaphragm in comparison with the unaffected side and the control.

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On fluoroscopic examination, severity of the involvement may be roughly estimated by the presence of paradoxic movement in relation to the strength of breathing. Its presence during quiet breathing signifies a severe degree of weakness of the diseased diaphragm in contrast to occurrence during deep inspiration or sniffing in a case of milder degree. An inspiratory excursion of the mediastinum toward the unaffected side observed in some patients implies a greater negative intrapleural pressure on that side during inspiration.

The contraction of the diaphragmatic musculature in response to the electrical excitation of the phrenic nerve merely indicates that both nerve and muscle are functioning to a certain extent. When there is partial involvement of either structure, the contraction response may be somewhat weaker but impossible to estimate accurately.

The technique of recording intragastric pressure with observation of respiratory variations (Schlippe's procedure, 5) is of no practical value for routine use because of its tedious process and the variable results obtained.

Case Report

S. P., a 35-year-old Thai housewife, was admitted to Siriraj Hospital on February 6, 1958, for severe hypersensitivity to para-aminosalicylic acid (PAS). Because her chest roentgenogram constantly showed a markedly high position of the left dome of the diaphragm (figure 1*A*, *B*), an anomaly of some sort was suspected. Her virtually negative history of illness except for some bouts of gastrointestinal disturbance, coupled with the physical findings of marked generally decreased movements of the left hemithorax, positive Hoover's sign, negligible rise of inspiratory tension of the left epigastrium, and the result of ulnar palpation of the back, led to the diagnosis of a high and weak diaphragm, possibly of a congenital origin.

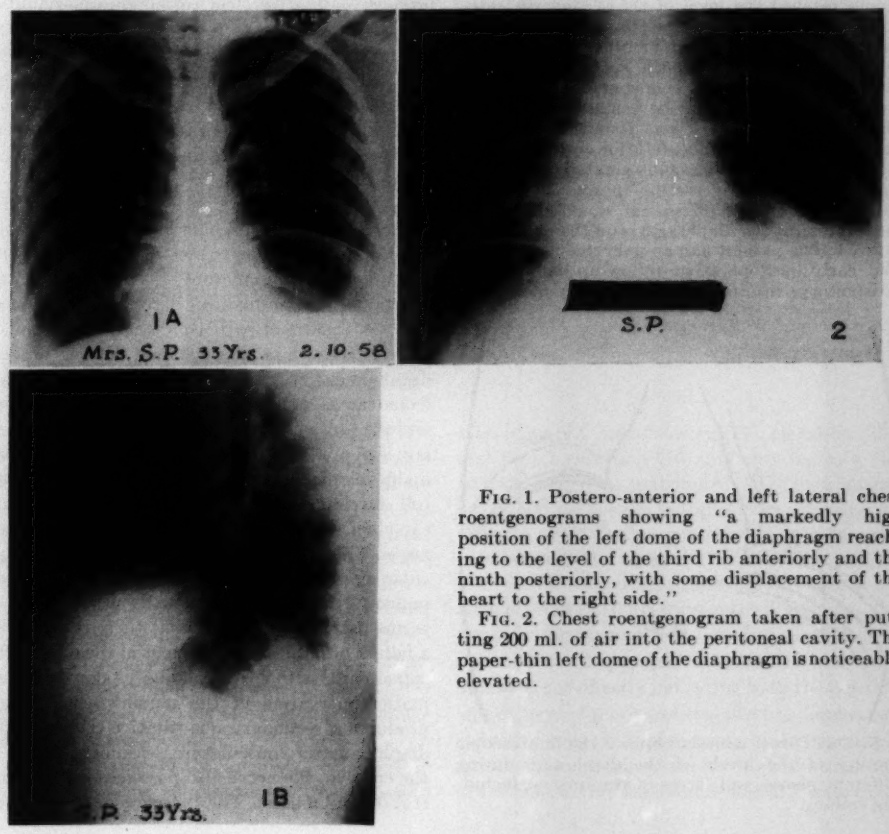

Fig. 1. Postero-anterior and left lateral chest roentgenograms showing "a markedly high position of the left dome of the diaphragm reaching to the level of the third rib anteriorly and the ninth posteriorly, with some displacement of the heart to the right side."

Fig. 2. Chest roentgenogram taken after putting 200 ml. of air into the peritoneal cavity. The paper-thin left dome of the diaphragm is noticeably elevated.

The results of all subsequent investigations confirmed the above suspicion. The thinness of the left leaf of the diaphragm was revealed by air in the peritoneal cavity (figure 2). The inspiratory fall of intragastric pressure (minus 14 mm. of water) seemed to correspond with the paradoxic movement of the left diaphragm and the mediastinal swing present on sniffing (figure 3). These findings are indicative of a moderately severe degree of weakness. The small left phrenic nerve seen on exposure for faradic stimulation furnished direct evidence of neural involvement feasible during life.

Hypoplasia of the left lower lobe of the lung with an abnormal positioning of a branch of the left upper lobe bronchus and an abnormally high position of the left kidney verified by bronchoscopy and pyelography, respectively, are associated anomalies which further strengthen the suspicion of congenital origin.

On March 18, 1959, an operation for repair of the abnormality and biopsy study was performed. At operation, the left diaphragm appeared as a relaxed translucent aponeurotic membrane; the cupola reached the level of the second rib anteriorly. The attachment was found at the usual level. There was no breach or rupture of the leaf. The serous linings on both surfaces were apparently normal. Only a sparse amount of muscle fibers was noticed near the costal attachments. The left phrenic nerve was found to be much smaller than usual. The left lower lobe of the lung was small, but it was fully aerated. The spleen and the left kidney were abnormally high in position. An area of 167 sq. cm. was excised from the left leaf of the diaphragm, and the edges were sutured. The patient had an uneventful recovery. The early postoperative follow-up study gave satisfactory results of the chest movements, diaphragmatic movements, and the level of the reconstructed diaphragm (figure 4*A*). On later follow-up observation, all of them have almost returned to the previous status (figure 4*B* and *C*).

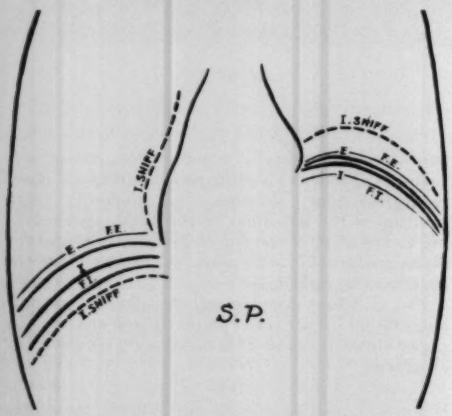

FIG. 3. Direct contact copy of the fluoroscopic picture of the levels of the diaphragm during different phases and forces of respiration, including sniffing.

Study of the excised portion of the diaphragm was done by one of the writers (S. S). Macroscopically, the patient's tissue appeared as a thin translucent membrane. No muscle was observed by macroscopic examination. Histologic study was performed by the method described in an earlier paper (6). All sections from the biopsy specimen revealed the absence of muscle tissue.

DISCUSSION

It is generally held that the surgical repair of the diaphragm should not be recommended for adult patients with no distressing symptoms. The present writers agree with Sanford (7) and De Bord and Giunta (8) that reconstruction of the diaphragm must be instituted as an emergency treatment for small children and adults with cardiopulmonary disturbance. Chin and Lynn (9), however, advise surgical correction in asymptomatic young patients with gross abnormalities. As the patient had good contraction response of the diaphragm to electrical excitation of the phrenic nerve and paradoxic movement only on sniffing, the reinforcement technique was not performed. The result of repair has, so far, not been beneficial.

In the present case, the formulation of the pathogenesis as "a primary neurogenic developmental failure leading to secondary aplasia of the diaphragmatic musculature" is based first on the constant relevant findings described in the majority of reports of pathologic study and, second, on embryonic data. The constant findings of maldevelopment of both nerve and muscle in the affected areas indicate the close relationship and exclude any chance coincidence. The absence of any sign of atrophy of either element implies that maldevelopment occurs quite early, that is, before full development. In the embryonic diaphragm, the chief nerve to the diaphragm is the phrenic nerve, which begins to send fibers to the premuscular masses of mesenchyma very early in developmental life (before the sixth week) (10). It seems natural to presume that there is primarily a failure of normal development of the cells of the spinal cord, with correspondingly poor diaphragmatic innervation of the phrenic nerve which produces a secondary quantitative defect of the diaphragmatic musculature, assuming that, in the embryo, the nerve also possesses trophic control on the muscle. The presence of underdevel-

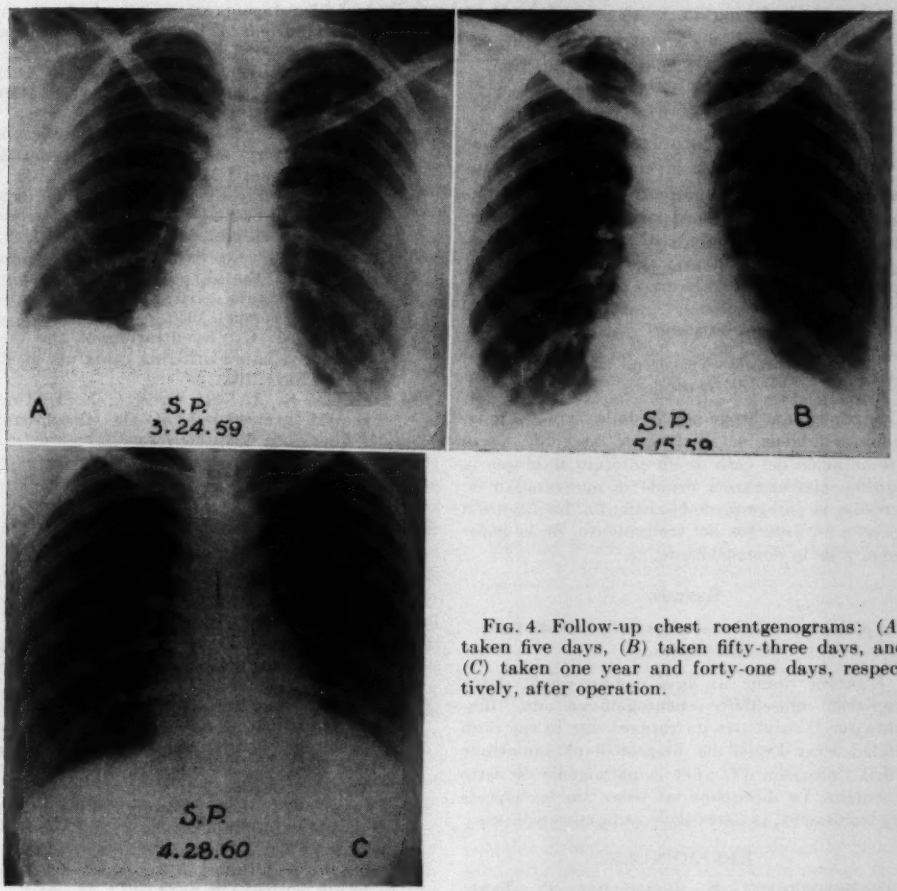

FIG. 4. Follow-up chest roentgenograms: (*A*) taken five days, (*B*) taken fifty-three days, and (*C*) taken one year and forty-one days, respectively, after operation.

oped muscle cells in one particular report (6) further strengthens the view of early developmental failure. Diaphragmatic innervations originate from two sources: the main supply from the phrenic nerve and small additional fibers from the lower intercostal nerve (11). Thus, when the periphery of the diaphragm is affected, the lower intercostal nerve fibers and the corresponding spinal cord cells should likewise be fewer in number than in normal cases. The histologic findings in the biopsy specimen of the present patient once again support the concept of congenital diaphragmatic muscular aplasia.

All of the names of this anomaly, i.e., eventration (3), dilatation or relaxation (12), idiopathic high-lying (13), insufficiency (14), elevation (15), and Petit's elevation (16), are only partially descriptive and none contributes to the understanding of the pathologic nature of the anomaly. In order to avoid further controversy on nomenclature between the congenital and acquired elevation of the diaphragm, and also between the anomaly and the herniation of the diaphragm, the precisely descriptive term "neurogenic muscular aplasia of the diaphragm" is used. This new term, although not directly indicating both the position and dynamic function of the affected diaphragm, conveys a wide range of meaning of structural and functional muscular weakness from the slightest to the most severe degree and clinically from cases

not detectable during life to those with marked thinning and elevation ("eventration") of the diaphragm with practically no dynamic function.

Summary

A short account of "neurogenic muscular aplasia of the diaphragm" is given, followed by a report of the case of one patient in whom diaphragmatic biopsy offered an opportunity to study the pathogenesis of the anomaly. The discussion was based on aspects of treatment, pathogenesis, and nomenclature.

Sumario

Aplasia Muscular Neurógena (Eventración) del Diafragma

Se ofrece una breve reseña de la "aplasia muscular neurógena del diafragma," seguida de la presentación del caso de un enfermo en el que la biopsia diafragmática brindó la oportunidad de estudiar la patogenia de la anomalía. La discusión se basa en aspectos del tratamiento, de la patogenia y de la nomenclatura.

Resume

Aplasie musculaire neurogènique (éventration) du diaphragme

L'auteur donne un bref compte-rendu d'une "aplasie musculaire neurogènique du diaphragme," suivi par un rapport sur le cas d'un malade chez lequel une biopsie diaphragmatique offrit l'occasion d'étudier la pathogénie de cette anomalie. La discussion est basée sur les aspects du traitement, la pathogènie et la nomenclature.

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Correspondence

PENICILLIN AND TUBERCLE BACILLI

To the Editor of the American Review of Respiratory Diseases:

Recently, Drs. Vaichulis and Doll[1] pointed out that penicillin is unstable in the Dubos medium used for culturing tubercle bacilli. Since all of the conventional media for growing these organisms contain anti-penicillin factors, they concluded that the inhibitory concentration for this drug against *Mycobacterium tuberculosis* had never been validly examined. With the kind assistance of Dr. Vaichulis, we have repeated as closely as possible the technique which they used, and have confirmed that the drug does disappear from the Dubos medium quite quickly. The fall in penicillin concentration in a similar medium in use in this laboratory (Middlebrook's 7H9 liquid) was also studied. Our figures for percentage loss of potency of 25 units of commercial penicillin G per ml. after 12, 24, 48, 72, 96, 120, and 168 hours of incubation at 37°C. in this medium were 0, 0, 0, 14, 46, 55, and 74 per cent, respectively.

In experiments reported recently,[2] which had been devised to study the time-concentration relationships of isoniazid and tubercle bacilli, a strength of 25 units of commercial penicillin G sodium (Glaxo) per ml. had been employed to assist in avoiding contamination of the 7H9 medium. Since the medium in contact with the tubercle bacilli was changed twice daily, it became important to be sure that penicillin in this strength was harmless, because under the particular experimental conditions no loss of penicillin would have occurred. Accordingly, using the same technique for changing the medium in which the bacilli were being incubated, this matter was investigated. Very small inocula of tubercle bacilli (6 drops of a 1:1,000,000 dilution of an eight-day-old Tween® culture) were exposed at 37°C. for six days to 7H9 medium containing 25 units of penicillin per ml. The penicillin was prepared from crystalline material once daily in buffer at pH6, kept at 4°C., and added to the medium only immediately before use. This drug medium was drained off every twelve hours and replaced with new drug medium. On the seventh day the bacilli were washed, subcultured on Löwenstein-Jensen medium, and the cultures were examined weekly for four weeks, after which colony counts were made. Appropriate control cultures without penicillin were carried through in the same way. Three strains were studied: H37Rv, a wild strain recently isolated from a newly admitted patient, and BCG.

The experiments were performed in duplicate and the duplicate colony counts are shown in table 1. The differences between tests and controls are within the limits of experimental error. This indicates that no bactericidal effect was produced. The colonies appeared at the second week in both tests and controls. When the inoculum itself was placed directly on the solid medium, growth was not observed until the third week. This shows that bacillary multiplication was satisfactory in the liquid medium under the conditions of two daily changes of medium. Therefore, because the colonies in tests and controls appeared to be the same size after the same incubation period, it is evident that no bacteriostatic effect was demonstrated either.

TABLE 1

INFLUENCE OF PENICILLIN ON TUBERCLE BACILLI

Colony Counts on Membrane Filters after 4 Weeks of Incubation on Löwenstein-Jensen Medium Subsequent to 6 Days' Exposure to a Penicillin-containing Medium

H37Rv		Wild Strain		BCG			
				Experiment 1		Experiment 2	
Tests	Controls	Tests	Controls	Tests	Controls	Tests	Controls
5	10	20	9	7	9	1	2
4	5	5	5	4	8	0	1

It is concluded that 25 units of penicillin per ml. in 7H9 liquid medium can be employed in culturing tubercle bacilli for at least six days, even under conditions which ensure no loss of the drug during this time, without influence on the growth of the bacilli.

A. RILEY ARMSTRONG
Hamilton Health Association
Hamilton, Canada

August 25, 1960

METABOLISM OF ISONIAZID

To the Editor of The American Review of Respiratory Diseases:

We are pleased to learn that a physicochemical procedure with high sensitivity and accuracy

[1] VAICHULIS, E. M. K., AND DOLL, J. P.: Amer Rev Resp Dis, 1959. *80*, 262.

[2] ARMSTRONG, A. R.: Amer Rev Resp Dis, 1960, *81*, 498.

has been devised for the measurement of the isoniazid molecule in plasma by Dr. John H. Peters.[1] We are also reassured to observe that application of this method of determination[2] of isoniazid has confirmed observations to the effect that para-aminosalicylic acid (PAS) can interfere with the metabolic modification of isoniazid to various derivatives in human subjects.[3-6] Although Dr. Peters has stated that "These (his) findings provide no support for the concept that therapeutic doses of PAS block acetylation or otherwise alter the metabolism of isoniazid in man,"[2] our interpretations of his data have led us to this opposite view.

Results presented by Dr. Peters in table 2 of his most recent paper[2] fairly identify his first five subjects as more or less "rapid inactivators" of isoniazid, and the last four as "slow inactivators" of this drug. With regard to the latter group, it is evident that a drug which interferes with biochemical modifications of another drug would have little or no effect on the plasma concentrations of that drug in individuals who do not significantly modify it. Thus the failure of PAS to modify measurably the plasma or serum levels of antimicrobially active isoniazid, as measured by microbiologic assay in individuals who have high serum levels of isoniazid without this drug (the "slow inactivators"), was already noted in the first publication on this subject.[3]

We have taken the liberty of applying conventional statistical methods of analysis (*t* test for paired variates) to some of Dr. Peters' results.[7] For the reasons stated above, our statistical analyses have been limited to the data recorded in the above-mentioned table[2] under "total hydrazides as isoniazid" in the plasma of the "rapid inactivators." The P-value for differences between the results obtained in these individuals given isoniazid plus PAS, and isoniazid alone, is less than 0.001, a highly significant difference, *statistically*. Thus his results, obtained by physicochemical assay, are not incompatible with our results as obtained by microbiologic assay.

The *biologic* significance of this unequivocal effect of PAS on the plasma concentrations of isoniazid has been discussed previously[6] and is beyond the scope of this letter.

Finally, for those who find our current microbiologic assay method for isoniazid[8] to be unsatisfactory in their hands, especially with regard to accuracy or reproducibility, we hope that this new method will be exploited.

GARDNER MIDDLEBROOK
WILLIAM F. RUSSELL, JR.
Division of Research and Laboratories
National Jewish Hospital at Denver
Denver, Colorado

September 14, 1960

[1] PETERS, J. H.: Amer Rev Resp Dis, 1960, *81*, 485.

[2] PETERS, J. H.: Amer Rev Resp Dis, 1960, *82*, 153.

[3] MANDEL, W., *et al.*: Proc Soc Exp Biol Med, 1956, *91*, 409.

[4] MORSE, W. C., *et al.*: Amer Practit, 1956, *7*, 1600.

[5] BELL, J. C., *et al.*: Amer Rev Tuberc, 1957, *76*, 152.

[6] MANDEL, W., *et al.*: J Clin Invest, 1959, *38*, 1356.

[7] We gratefully acknowledge Dr. Solbert Permutt's calculations and advice in this matter.

[8] MIDDLEBROOK, G., *et al.*: Acta Tuberc Scand, 1960, *38*, 66.

Books

MIRAGE OF HEALTH. Utopias, Progress, and Biological Change. By René Dubos. Pp. 236, Harper & Brothers, New York, 1959. Price, $4.00.

This little book is volume 22 of a series called "World Perspectives," which are planned and edited by Ruth Nanda Anshen. Dr. Dubos has divided his text into eight chapters, as follows: The Garden of Eden; Biological and Social Adaptation; Struggle and Partnership in the Lving World; Environment and Disease; Hygeia and Aesculapius; Social Patterns of Health and Disease; Effects of Disease on Populations and on Civilization; Utopias and Human Goals.

In this series of essays Dr. Dubos emphasizes the point that there can be no Garden of Eden where man is perfectly happy, healthy, and free from toil and sorrow. "Health and happiness cannot be absolute and permanent values, however careful the social and medical planning. Fitness requires never-ending efforts of adaptation to the total environment, which is ever changing."

The author points out that the characteristics of the total environment determine in a large measure the types of disease most prevalent in a community. The appearance of new antibacterial drugs gave rise to the illusion that the age-old problem of infection had been solved. It is true that chemotherapy has transformed the practice of medicine, but there is no reason to believe that antimicrobials "spell the conquest of microbial disease."

The remarkable fact is that the greatest strides in health improvement have been achieved in the field of diseases that responded to social and economic reforms after industrialization.

But changes in our physical and social environment have brought about an increase in prevalence of other diseases with which we are not, as yet, prepared to cope.

The essay on social patterns of health and disease is perhaps the most challenging of the series.

"The modern state is predicated on the assumption that happiness is best reached through freedom from want and from struggle." But this concept of a Garden of Eden is not achievable or desirable, for Paradise is a static concept, whereas life is a dynamic process.

"The Earth has never been a Garden of Eden, but a Valley of Decision where resilience is essential to survival. Man has elected to fight not necessarily for himself but for a process of emotional, intellectual, and ethical growth that goes on forever. To grow in the midst of danger is the fate of the human race, because it is the law of the Spirit."

This brief review of these essays by Dr. Dubos gives only a taste of the flavor of his thinking. It is not a reviewable book. It should be read slowly, carefully, and in its entirety. It is a book that should be kept in a convenient place to read again from time to time.

WILSON G. SMILLIE
Newfane, Vermont

HANDBOOK OF CIRCULATION. Edited by Dorothy S. Dittmer and Rudolph M. Grebe. Pp. 393, W. B. Saunders Company, Philadelphia, London, 1959. Price, $7.50.

The National Research Council has published another of its popular handbooks, this one devoted to the circulation. As with previous publications of this nature in the past decade, the present volume contains an impressive mass of information of almost general usefulness.

The contents are divided into a total of seventeen sections, beginning with the circulatory anatomy and chemical and physical properties of the vascular system, and extending through a variety of physiologic and pathologic data. To the mammalian physiologist, it is pleasantly surprising to find a section on the insect heart as well as more than thirty pages devoted to the circulatory physiology of plants! Where else would one find a volume of use to the cardiac surgeon, the plant pathologist, and the entomologist?

The sections on drug effects, chemistry, and pathologic conditions seem of particular usefulness to clinical physiologists. Pulmonary vascular physiology and pathology, in particular, are covered extensively. Certain of the sections, such as those on ionizing radiation, high altitude, and other environmental circumstances will doubtless prove of reference value to "space medicine physiologists," while the formulas and equations collected in the appendix should be useful also to students.

As with all handbooks, this one has certain inadequacies and omissions. The sections on influence of pathologic conditions on the cardiovascular system contain little or nothing with regard to obesity, an unfortunate omission. Electrocardiograms are discussed in extensive detail with regard to certain selected features such as

r-wave amplitudes, et cetera, including instructions on how to take an electrocardiogram; yet other equally important portions of the complexes are not mentioned at all. It would have been better to omit this section completely. Plates 6 and 7, depicting the gross anatomy of the arteries and veins, could also have been left out without detracting from the value of the book. Difficulty is encountered especially when attempting to locate any particular drug in the volume, other than the more common ones listed in the index. The anatomic features of the micro-circulatory system have been almost completely omitted. Further attention in this area would have greatly increased the value of the handbook.

In spite of these defects, one seldom finds a single volume of such general usefulness. It should be a part of the library in any laboratory engaged in vascular research in these fields.

Richard E. Lee
Department of Medicine
New York Hospital
New York, New York

Preventive Medicine. Edited by Herman E. Hilleboe and Granville W. Larimore. Pp. 731, W. B. Saunders Company, Philadelphia, 1959. Price, $12.00.

The editors have chosen to present their subject in terms of what can be done, on the one hand, to prevent the occurrence of illness and injury and, on the other, to prevent or retard progression of disease and disability and to minimize its effects upon the patient's way of life.

The point is made that the traditional practice of medicine actually contributes a good deal to this latter variety of preventive medicine, but that public demand is building up for something more than care of actual illness. The physician is expected to provide a wide variety of preventive services and to make discriminating use of community resources for health protection and promotion just as he now employs therapeutic resources traditionally associated with the practice of medicine. Whether or not he realizes it, today's man in practice has been assigned a key role in his community's organized effort to promote its health.

The stated purpose of this book is to point out to the physician who is willing to meet this challenge the opportunities which exist for him to render effective preventive services to his patient during the course of his day-to-day practice, and to provide him with information he needs in order to do this.

The material for this book has been selected from among numerous articles, seminars, and lectures prepared by members of the New York State Department of Health for use in the Department's active teaching and training program. The topics covered include most of those usually discussed in a text of this kind, with the exception of community mental health, which receives practically no mention. In addition, some relatively new areas of public health action are presented, such as civil defense, protection against radiation, and adult health maintenance. Several interesting chapters are devoted to this latter question in which there is detailed discussion of the role of periodic physical examinations in a program of adult health promotion and of results obtained by screening examinations specifically designed to uncover certain chronic diseases.

Although the quality of the various contributions is somewhat uneven, the work as a whole is a useful addition to the reference shelf of texts dealing with preventive medicine in public health.

Ann P. Kent
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Flushing–Corona Health Districts
Jackson Heights, New York

Automatic Ventilation of the Lungs. By William W. Muschin, L. Rendell-Baker, and Peter W. Thompson. Pp. 349, Charles C Thomas, Springfield, Illinois, 1959. Price $10.50.

This skillfully composed volume is written by and for anesthesiologists but, as the problem of respiratory failure becomes numerically more significant, the importance of more than casual understanding in this field increases for the internist. The first third of the book discusses physiologic aspects of controlled ventilation in a concise and practical manner. The relationship between compliance, resistance, flow rate, alveolar pressure, and mouth pressure is clearly illustrated. The differences between spontaneous and controlled respiration and the possible harmful effects of the latter are discussed. Knowledge of these effects, which include absence of the "thoracic pump," tamponading action of positive intrapulmonary pressure at a time when it normally is subatmospheric, and obstruction to pulmonary blood flow due to the capillary-collapsing effect of positive intra-alveolar pressure, may well be more important to the internist treating patients with pulmonary hypertension, low cardiac output, and heart failure than to the anesthesiologist dealing with good operative risks. The importance of mean as opposed to peak intrapulmonary pressure is clearly stated, and factors increasing the one and the other are graphically illustrated.

In considering the physical aspects of the operation of an automatic ventilator, its action is di-

vided into four parts: inspiratory phase, change-over from inspiration to expiration, expiratory phase, and change-over from expiration to inspiration. Discussing the first, flow generators in which flow is constant and pressure changes depend on the physical characteristics of the recipient lung are differentiated from pressure generators in which pressure is determined by the assisting mechanism and flow depends on the physical characteristics of the patient's lung. Flow generators are further subdivided into those with constant flow and those with sine-wave flow, and the reflection of this difference in air flow, lung volume, alveolar pressure, trans-airway pressure gradient, and mouth pressure is illustrated with simple graphs.

Similarly, the physical effects of various flow, volume, and pressure limitations on expiration are clearly discussed. Change-over between respiratory phases can be triggered by volume change, pressure change, or passage of a time interval; these characteristics of the mechanism all have differing physiologic consequences. Actual ventilation achieved is presented as the resultant of characteristics of the recipient lung and of the respirator. A machine which performs well in one circumstance may be poorly suited to another.

The last two-thirds of the book are devoted to a detailed mechanical and functional analysis of all types of mechanical ventilators in use at the time of publication, complete with schematic diagrams.

The administration of mechanical devices for assisting respiration is a major medical maneuver which is, unfortunately, too often done in a noncritical fashion. Certainly the clinician should be aware of the characteristics of the machine in question and their physiologic consequences. I know of no other easy source for this important information.

Roger M. Des Prez
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New York, New York

Robert Koch—Briefe an Wilhelm Kolle. Pp. 67, Georg Thieme Verlag, Stuttgart (in U. S. and Canada, Intercontinental Medical Book Corporation, New York), 1959. Price, $4.30.

Robert Koch died of a coronary occlusion on May 27, 1910. On the occasion of the fiftieth anniversary of his death, Professor Kurt Kolle, son of one of his closest associates, has edited a number of private letters which have not been published previously.

The first part of this small volume contains several letters written to his aunt while Koch was studying medicine at the University of Göttingen which furnish interesting information about the insight thus far into the later development of this extraordinary man. The second part consists mainly of letters written to Professor Wilhelm Kolle, the father of the editor, during the time that Robert Koch was engaged in his work on South African cattle plague.

A great many intriguing details can be obtained from these letters, among others that Koch himself considered his work on malaria the most important contribution of his scientific career. There is an interesting letter replying to a request by Kolle for promotion and a salary increase at the Institute for Infectious Diseases in Berlin, which had been founded by Robert Koch and was later named after him. The positions here were so poorly paid because of the meager budget the Institute was allowed that Koch himself could consider them nothing but a jumping board for gifted associates to obtain leading—and of course better remunerated positions—in the academic field. It seems that fifty years later a similar letter would not be considered unusual.

This small booklet is not meant to introduce the reader into the life of Robert Koch, but it will be welcomed warmly by every scholar who desires additional information about the life and personality of the man.

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Department of Pathology
New York Hospital—
Cornell Medical Center
New York, New York

BOOKS RECEIVED

Viral Infections of Infancy and Childhood. Symposium No. 10, Section on Microbiology, The New York Academy of Medicine. Edited by Harry M. Rose. Pp. 244, Hoeber-Harper, New York, 1960. Price, $8.00.

The Heart in Industry. Edited by Leon J. Warshaw. Pp. 677, Paul B. Hoeber, Inc., New York, 1960. Price, $16.00.

Cellular Aspects of Immunity. Ciba Foundation Symposium. Edited by G. E. W. Wolstenholme and Cecilia M. O'Connor. Pp. 495, Little, Brown & Company, Boston, 1960. Price, $10.50.

Current Drug Handbook, 1960–61. By Mary W. Falconer and H. Robert Patterson. Pp. 164, W. B. Saunders Company, Philadelphia, London, 1960. Price, $2.50.

American Thoracic Society

Medical Section of the National Tuberculosis Association

Tuberculosis Control Programs for Nurses, Nursing Students, and Hospital Personnel

A Statement of the Committee on Therapy

A tuberculosis control program is necessary for all hospital employees who are in frequent contact with patients. Such personnel, including doctors, nurses, nursing students, medical students, ward aids, technicians, social workers, and others, may be exposed to greater risk of infection in so-called nontuberculous areas, i.e., general medical, surgical, and psychiatric wards, than in recognized isolation wards. Therefore, the following recommendations apply to these personnel wherever they work in a hospital.

A tuberculosis control program has two phases: the prevention of infection and the early detection of infected individuals.

Detection of infected personnel: At the time of employment (or on enrollment of medical and nursing students), the reaction to an intradermal tuberculin test of 5 TU (tuberculin units) should be determined and a chest film obtained for all personnel. Fourteen- by seventeen-inch films have certain advantages over microfilms, both in detecting existing minimal lesions and in serving as baselines for comparison with subsequent roentgenographic examinations. Tuberculin patch tests are not a satisfactory substitute for the recommended intradermal tuberculin tests. Also, documentary evidence of a recent tuberculin test or chest roentgenogram is not always reliable and, therefore, these tests should be performed by the agency or individual in the hospital or school responsible for tuberculosis control.

In personnel with positive reactions to their initial tuberculin tests, 14- by 17-inch chest films should be repeated in three months and thereafter at six-month intervals. Tuberculin tests need not be repeated. Chest roentgenograms should be compared with previous examinations and any change considered an indication for complete medical evaluation.

In tuberculin-negative personnel, the tuberculin test, when feasible, should be repeated at six-month intervals. Conversion to a positive reaction is evidence of a recent tuberculous infection and indicates the need for complete medical evaluation. The management of an adult tuberculin converter has been dealt with in a previous report by this committee.[1]

Prevention of infection of personnel: Detection of patients with active tuberculosis is of prime importance in reducing the incidence of infection of hospital personnel. This is accomplished satisfactorily only by obtaining routine chest roentgenographic examinations for all adult hospital admissions. In addition, proper instruction and hygienic precautions should be provided for those patients who might tend to disseminate tubercle bacilli by uncontrolled cough, expectoration, or sneezing.

The use of BCG vaccine for persons failing to react to the tuberculin test may be considered in certain circumstances, especially in advance of an anticipated exposure to sources of infection. When used, this vaccine must be administered at least six weeks prior to the anticipated exposure in order to ensure that tuberculin conversion is due to vaccination and not to an acquired tuberculous infection. Indications for the use of BCG have been considered in a previous ATS statement.[2]

Thomas B. Barnett
Benjamin Burrows
Edward Dunner
John R. Durrance
Attilio D. Renzetti, Jr.
Herbert Schmidt
William W. Stead
Donald L. Paulson, *Chairman*

[1] American Trudeau Society: Treatment of the adult tuberculin converter: A statement of the Committee on Therapy, Amer Rev Resp Dis, 1960, *81*, 443.

[2] American Trudeau Society: Statement on BCG, Amer Rev Tuberc, 1949, *60*, 681.

Obituaries

Sidney A. Slater

1884–1959

Becoming a victim of pulmonary tuberculosis soon after graduation from medical school, S. A. Slater thereafter devoted his life and work to antituberculosis activities until residual pulmonary fibrosis and emphysema caused his death on June 12, 1959. He possessed unusual initiative and energy, had a clear vision of the far-off tuberculosis eradication goal and, best of all, was richly endowed with common sense.

From the outset he had a keen awareness of the strategic importance of general practitioners and of their value as allies in the battle against the tubercle bacillus, derived in part from his own experience of two years in general practice and in part from Dr. Trudeau's statement about general practitioners in the first presidential address of the National Tuberculosis Association. Therefore, soon after assuming the medical directorship of the Southwestern Minnesota Sanatorium in January, 1919, he personally kept in touch with each physician in the eight counties of his district, with the result that every physician in the district became well informed on all aspects of tuberculosis and followed their leader who had set eradication as his goal.

His patients always received the most modern treatment. Dr. Slater was the first person to institute artificial pneumothorax in this country to control tuberculosis in a pregnant woman (1918). Those in need of chest surgery were transferred to other hospitals under the care of first-class chest surgeons and later returned to him as convalescent patients. When antituberculous drugs became available, he promptly employed them. Nothing known to be of value in the treatment of tuberculosis was omitted.

In 1924, Dr. Slater startled the tuberculosis world by reporting before the annual meeting of the National Tuberculosis Association held in Atlanta, Georgia, that he had tested 1,654 rural school children with tuberculin and that only about 10 per cent reacted. However, among those from homes where contagious tuberculosis had existed, 80 per cent reacted. This report caused considerable furor because of the extant belief that 90 to 95 per cent of children were infected by the age of fifteen years. There were those who contended that Slater had not used fresh potent tuberculin. Others said the Pirquet scratch test was inferior, quite forgetting that Ghon's classical post-mortem work published in 1912 was based on this method. It was said that, if he had used the intracutaneous method, the percentage of reactors would have been what most everyone thought it should be. He later adopted this method, but found that it did not produce a significantly larger number of reactors.

In the 1920's, he observed that the clinical cases admitted to his sanatorium were derived from the backlog of tuberculin reactors. Recognizing that eradication must include all pathogenic types of tubercle bacilli, he became an ardent supporter of the bovine tuberculosis eradication campaign. All of the counties in his district became officially accredited as modified tuberculosis-free areas as early as 1934.

The measure of Dr. Slater's success in tuberculosis control was demonstrated by the fact that in 1956 only one person died of tuberculosis in his district, which then had a population of 230,000, whereas 67 had died in the eight original counties in the year 1919 when he began his work. Paradoxically, this success led to the greatest disappointment of his life. Upon his attainment of the legal retirement age in November, 1957, his sanatorium was closed because of the accumulation of vacant beds. Inasmuch as 25 per cent of the population of the district still had positive tuberculin reactions, his vision of total eradication was blasted, and in its place he visualized tuberculosis returning in full force as the decades pass. Had he survived a few months longer, he no doubt would have derived great satisfaction from the adoption by the Arden House Conference of the concept of eradication as the ultimate goal.

Throughout his life Dr. Slater was an active worker in all of the tuberculosis organizations. He was the first president of the Minnesota Sanatorium Association (1922), a founder (1925) and president (1935) of the Minnesota Trudeau Society, president for four years of the Minnesota State Tuberculosis Association, and for twenty-nine successive years was his state's representative on the Board of Directors of the National Tuberculosis Association.

Dr. Slater received numerous honors for his achievements in tuberculosis control, including the Dearholt medal from the Mississippi Valley Conference on Tuberculosis, a special certificate from the Minnesota State Medical Society, and an honorary degree from his alma mater, the University of Richmond.

J. Arthur Myers

Charles Howard Miner

1868–1959

Dr. Charles H. Miner, one of the founding members of the National Tuberculosis Association,

died at his home in Wilkes-Barre, Pennsylvania, on July-12, 1959, at the age of ninety-one. He was born in Wilkes-Barre on July 5, 1868. His forebears came to Wyoming Valley with the early settlers from Connecticut, and his marriage to Grace Lee Shoemaker united two families of Colonial days, old in the history of northeastern Pennsylvania.

Dr. Miner received his preliminary education at the Wilkes-Barre Academy, which is today the Wyoming Seminary Day School. He was graduated from Princeton University with a Bachelor's degree in 1890, and his M.D. degree was awarded by the University of Pennsylvania Medical School in 1893. After an internship at the Presbyterian and further studies at St. Agnes and Children's hospitals in Philadelphia, Dr. Miner pursued graduate studies in Heidelberg and Vienna. He entered the private practice of medicine in 1896 in Wilkes-Barre, Pennsylvania. His early interest in tuberculosis led him in 1902 to establish a tuberculosis clinic, among the first to be established in the Commonwealth of Pennsylvania, subsequently taken over by the State as a model for the development of the state-wide program of tuberculosis clinics. From his original clinic has grown the Kirby Memorial Health Center.

Dr. Miner was appointed Medical Director of Luzerne County in 1907, and served in this capacity until he was named Secretary of Health for the Commonwealth by Governor Gifford Pinchot in 1923, in which position he served for a term of four years, thereafter returning to his duties in Luzerne County. In addition to his work in the official health agencies, he was an outstanding leader in the field of voluntary health agencies. He founded the Wyoming County Tuberculosis and Health Society and served on the Board of the Pennsylvania Tuberculosis and Health Society from 1908 to 1952, and was its president in 1927–1928. He was honorary vice-president from 1952 until the time of his death.

In 1925, Dr. Miner sponsored a meeting that resulted in the organization of the Pennsylvania Public Health Association. In regard to his Public Health vision, it seems fitting to quote from a tribute paid him in 1941. "He was among the members of the medical profession who years ago caught the vision of Public Health Services as a means of combatting preventable disease and safeguarding and promoting the health of the people."

Dr. Miner served in the Army Medical Corps in the Spanish-American War and examined recruits in both World War I and World War II.

He was a member of many medical organizations, notably the American Clinical and Climatological Association, to which he was elected in 1909. He was a Fellow of the American College of Physicians and the Philadelphia College of Physicians. He was a member of the National Tuberculosis Association and the American Sanatorium Association. He was certified by the American Board of Internal Medicine. One could go on and on enumerating Dr. Miner's medical accomplishments, but no comment would be complete without some statement as to the man. This can best be done by quoting from a tribute paid him by Dr. Frank A. Craig on the occasion of his being named honorary vice-president of the Pennsylvania Tuberculosis and Health Society. "A man of high and noble character, a gentleman of the first order, a greatly valued friend and supporter of the Pennsylvania Tuberculosis and Health Society and of the entire voluntary tuberculosis organization, a beloved physician and one who devoted his long life to serving his fellow human beings." No truer or finer tribute could be paid to anyone.

Dr. Miner is survived by his wife, a son, Charles H. Miner, Jr., a prominent attorney in Wilkes-Barre, and a daughter, Stella Mercer Shoemaker Miner.

DAVID ALEXANDER COOPER

NOTICES

The following dates and locations of the next four annual meetings of the National Tuberculosis Association and the American Thoracic Society have been selected and approved:

1961—Cincinnati, Ohio—May 21–26
1962—Miami Beach, Florida—May 20–25
1963—Denver, Colorado—May 12–17
1964—New York, New York—May 24–29

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Edward J. Welch, M.D.
1101 Beacon Street
Brookline 46, Massachusetts

ERRATUM

Due to printer's error, page *numbers* 461 and 462 have been repeated in the October issue of the *Review*, although already used in the September issue. The duplication of these pages is accounted for in the index by the addition of *Sept.* or *Oct.* for the entries involved.

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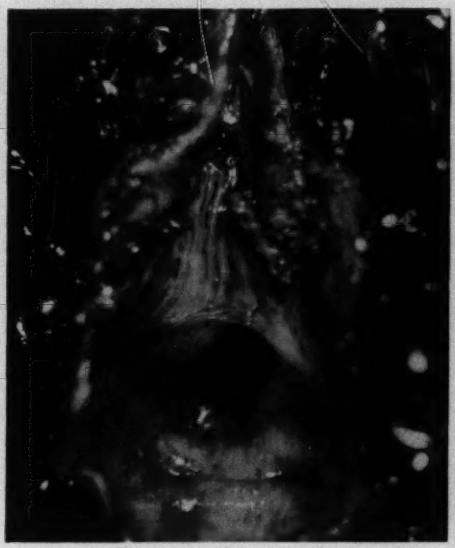

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INTERNATIONAL SYMPOSIUM OF THE DEBORAH SANATORIUM AND HOSPITAL

Philadelphia, Pennsylvania, November 20, 21, 22, 1958

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WANTED: Full time physician for new 300 bed tuberculosis division of Baltimore City Hospitals. Associated with two medical schools for teaching purposes with opportunity for research. Complete surgical and pathological facilities. Please address inquiries to Director of Personnel, Baltimore City Hospitals, Baltimore 24, Maryland.

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The Behavior of Pulmonary Tuberculous Lesions

by E. M. MEDLAR, M.D.

240 pages *43 illustrations in color*

In this monograph, published originally as a supplement to the March issue of The American Review of Tuberculosis and Pulmonary Diseases, now known as the American Review of Respiratory Diseases, the author has brought together in these eleven comprehensive sections the results of a lifetime of observation and interpretation.

I. Statement of the Problem.
II. The Experimental Approach.
III. Necropsy Studies of Human Pulmonary Tuberculosis.
IV. Preliminary Discussion of Resected Specimens.
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THE AMERICAN REVIEW OF RESPIRATORY DISEASES

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AMERICAN REVIEW OF RESPIRATORY DISEASES

Audience Profile

An analysis based on every tenth name of our United States subscription list checked against the *AMA 1958 Directory* to insure proper *geographical coverage*

Total Circulation	8,128
United States	4,651
Canada	250
Foreign and Miscellaneous	3,227

PHYSICIANS

SPECIALTY

	No.	%
No specialty	50	11.46
Starred pulmonary specialist	84	19.27
Pulmonary specialist	24	5.50
Starred internist	85	19.49
Starred surgeon	31	7.10
Surgeon	3	.68
Starred thoracic surgeon	27	6.19
Starred radiologist	7	1.60
Starred public-health specialist	5	1.14
Starred pathologist	3	.68
Starred pediatrician	3	.68
Starred otologist	2	.45
Starred and unstarred specialists in allergy, bacteriology, gastroenterology, hospital administration, industrial medicine, OALR, OBG, orthopedic surgery, physical medicine—one each	9	2.06
No information	17	3.89
	350	80.19
Institutional subscribers	86	19.72
	436	99.91

MAJOR PROFESSIONAL INTEREST

	No.	%
General private practice	22	5.04
Primary interest in internal medicine and pulmonary diseases	170	38.99
GP with interest in pulmonary diseases	24	5.50
Physician in advanced training	17	3.89
Full-time hospital staff	76	17.43
Full-time medical-school staff	7	1.60
Hospital administration	2	.45
Special research	1	.22
General public-health practice	10	2.29
Retired	3	.68
Not in practice	1	.22
No information	17	3.89
	350	80.19
Institutional subscribers	86	19.72
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AGE OF PHYSICIAN

	No.	%
No information	17	3.89
No age given	1	.22
30–34	10	2.29
35–39	55	12.61
40–44	58	13.30
45–49	50	11.46
50–54	51	11.69
55–59	37	8.48
60–64	35	8.02
65 and over	36	8.25
	350	80.21
Institutional subscribers	86	19.72
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POPULATION

	No.	%
Under 10,000	56	12.84
10–25,000	39	8.94
25–50,000	44	10.10
50–100,000	38	8.71
100–200,000	32	7.33
200–500,000	48	11.00
500–1,000,000	39	8.94
Over 1,000,000	54	12.38
	350	80.24
Institutional subscribers	86	19.72
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BREAKDOWN OF INSTITUTIONAL SUBSCRIPTIONS

Federal Hospital		General Hospital		Special Hospital		Laboratory		Medical Schools		College University		Society Library		Population	
3		2		5		2		0		1		0		Under 10,000	
3		2		1		0		0		1		0		10–25,000	
0		2		4		0		3		2		1		25–50,000	
1		1		1		0		1		2		2		50–100,000	
2		0		1		1		0		0		2		100–200,000	
3		6		0		0		0		1		4		200–500,000	
4		3		2		0		0		1		0		500–1,000,000	
2		4		1		1		2		0		6		Over 1,000,000	
18	4.12%	20	4.58%	15	3.44%	4	.91%	6	1.37%	8	1.82%	15	3.44%	86	19.72%

Percentages figured only to second decimal point

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with closure down the side

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- Four (4) times faster than Mantoux.
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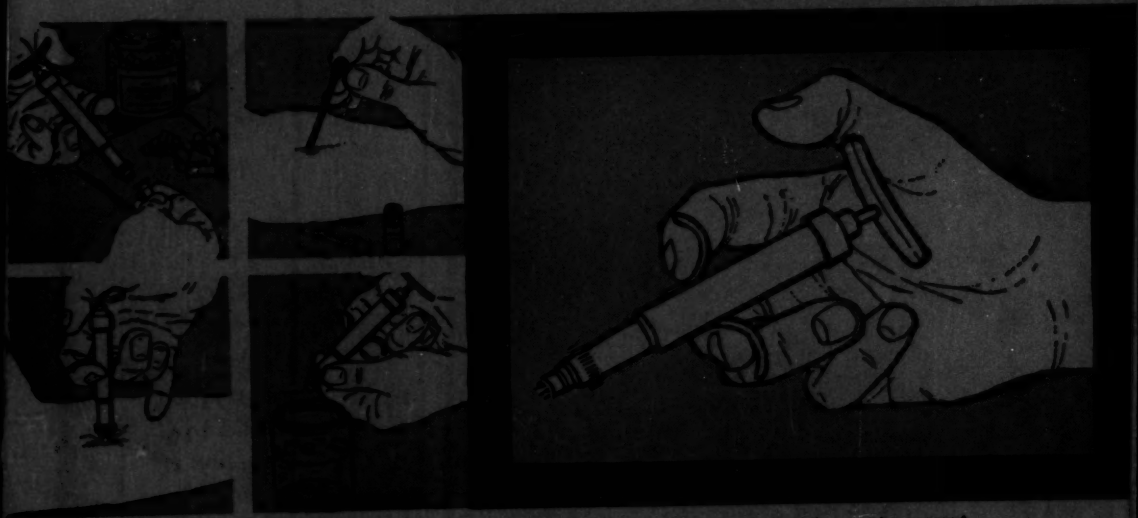

Procedure for STERNEEDLE TUBERCULIN TEST:

Top, left...Slip needle cartridge on gun with plastic applicator. Remove applicator.

Top, right...Place a drop of tuberculin PPD on cleansed forearm.

Bottom, left...Place gun on drop of tuberculin PPD. Pressure and release of handle automatically triggers and retracts the needles.

Bottom, right: Disposable needle cartridges and applicators may be washed and sterilized for reuse, if desired.

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